Allozyme and chromosomal Polymorphism in a laboratory Population of Drosophila subobscura.

Dissertation

zur Erlangung des Grades eines Doktors der Naturwissenschaften

dem Fachbereich Biologie
der Eberhard - Karls - Universität zu Tübingen

vorgelegt von

Michelle POULIN - ALFELD geb. Poulin

aus Redwood City, California

1978

78 511

UNIV.
LEIDEN
BIBL.

Allozyme and chromosomal Polymorphism in a laboratory Population of *Drosophila subobscura*.

D i s s e r t a t i o n

zur Erlangung des Grades eines Doktors der Naturwissenschaften

dem Fachbereich Biologie
der Eberhard-Karls-Universität zu Tübingen

vorgelegt von

Michelle POULIN-ALFELD geb.Poulin
aus Redwood City, California

1978

Tag der mündlichen Prüfung: 1.Dez. 1978

Dekan: Prof.Dr.F.Oberwinkler

1. Berichterstatter: Prof.Dr.D.Sperlich

2. Berichterstatter: Prof.Dr.W.Seyffert

UNIV.
LEIDEN
BIBL.

Acknowledgements

I am indepted to my supervisor, Professor D. Sperlich for his helpful comments and guidance in the research work described in this dissertation. The technical assistance of Dr. W. Pinsker with the electrophoresis is greatly appreciated , as is the assistance in maintaining the fly cultures given by D. Armbruster-Pinsker.

TABLE OF CONTENTS

Chapter one - Introduction 1

1.1 Genetic polymorphism 1

1.2 Enzyme polymorphism 1

1.3 Chromosomal polymorphism 2

1.4 Stable versus flexible chromosomal polymorphism 3

1.5 Purpose of this study 5

Chapter two - Materials and Methods 6

2.1 Study organism 6

2.2 Trapping of Drosophila 6

2.3 Maintenance of flies 8

2.4 Establishment and maintenance of cage populations 10

2.5 Sampling procedure 12

2.6 Chromosomal analysis 13

2.7 Enzyme analysis 13

Chapter three - Results 26

3.1 Enzyme analysis 26

3.2 Chromosomal analysis 26

3.3 Association analysis 27

Chapter four - Discussion 93

4.1 Enzyme analysis 93

4.1.1 Enzyme polymorphism: the natural population 93

4.1.2 Enzyme polymorphism: the cage populations 94

4.2 Chromosomal analysis 98

4.2.1 Chromosomal polymorphism: the natural population 98

4.2.2 Chromosomal polymorphism: the cage populations 103

4.3 Association analysis 112

4.3.1 Association between enzyme alleles 112

4.3.2 Associations between enzyme alleles and chromosomal arrangements 116

4.3.3 Associations between chromosomal arrangements 118

Chapter five - Conclusions 120

5.1 Enzyme polymorphism in nature 120

5.2 Stability of enzyme polymorphism 120

5.3 Chromosomal polymorphism in nature 120

5.4 Stability of chromosomal polymorphism 122

5.5 Enzyme allele interactions 124

5.6 Chromosomal arrangement interactions 124

5.7 Associations between enzyme alleles and chromosomal arrangements 125

5.8 Summary 126

Appendix 128

I Statistical analysis of data 128

II Chromosome inversions 131

Bibliography

LIST OF DIAGRAMS

2.7 Siphoning Exhauster

2.2 Species determination by starch electrophoresis

2.3 Arrangement of gels

2.4 Gel cutting apparatus

2.5 Allele determination - α Gpdh-3

2.6 Allele determination - Mdh-2

2.7 Allele determination - Idh

2.8 Allele determination - Me and Pgm

LIST OF TABLES

2.1 Representative flora at collection sites

2.2 List of chemicals

2.3 List of apparati

3.1 Alleles found for five enzyme systems

3.2 Original frequency of enzyme alleles

3.3 Relative frequencies of enzyme alleles - Cage I

3.4 Relative frequencies of enzyme alleles - Cage II

3.5 Relative frequencies of enzyme alleles - Cage III

3.6 Relative frequencies of enzyme alleles - Cage IV

3.7 Chi square values α Gpdh-3

3.8 Chi square values - Me

3.9 Chi square values - Mdh-2

3.10 Chi square values - Idh

3.11 Chi square values - Pgm

3.12 Frequency of chromosome A structural types

3.13 Frequency of chromosome I structural types

3.14 Frequency of chromosome O structural types

3.15 Frequency of chromosome U structural types

3.16 Frequency of chromosome E structural types

3.17 Chi square values: Chromosomes A, I, E

3.18 Chi square values: Chromosomes O, U

3.19 Larval enzyme allele frequencies - Mdh-2, Me

3.20 Larval enzyme allele frequencies - Idh, Pgm

3.21 Chi square values - larval enzyme alleles

3.22 Frequency heterozygotes for alleles of enzyme systems

3.23 Individuals heterozygous for various numbers of enzyme loci

3.24 Enzyme allele combinations: Mdh-2/Me-Interchromosomal

3.25 Enzyme allele combinations: Mdh-2/Idh-Interchromosomal

3.26 Enzyme allele combinations: Idh/Me-Interchromosomal

3.27 Enzyme allele combinations: Idh/Pgm-Interchromosomal

3.28 Enzyme allele combinations: Pgm/Mdh-Interchromosomal

3.29 Enzyme allele combinations: Pgm/Me-Interchromosomal

3.30 Mdh/Pgm allele combinations: Sample: R. Initial

3.31 Mdh/Pgm allele combinations: Sample: I_4

3.32 Mdh/Pgm allele combinations: Sample: II_4

3.33 Mdh/Pgm allele combinations: R. Initial and I_4

3.34 Mdh/Pgm combination: Original adult flies

3.35 Mdh/Pgm combination: Adult I_4 sample

3.36 Frequencies of heterokaryotypic individuals

3.37 Individuals heterozygotes for various numbers of chromosomes

3.38 Inversion combinations: Chromosome I and U

3.39 Inversion combinations: Chromosome I and E

3.40 Inversion combinations: Chromosome I and O

3.41 Inversion combinations: Chromosome U and O

3.42 Inversion combinations: Chromosome U and E

3.43 Inversion combinations: Chromosome O and E

3.44 Inversion - enzyme allele - combinations: Chromosome I/Mdh-2

3.45 Inversion - enzyme allele - combinations: Chromosome I/Me

3.46 Inversion - enzyme allele - combinations: Chromosome I/Idh

3.47 Inversion - enzyme allele - combinations: Chromosome I/Pgm

3.48 Inversion - enzyme allele - combinations: Chromosome O/Mdh-2

3.49 Inversion - enzyme allele - combinations: Chromosome O/Me

3.50 Inversion - enzyme allele - combinations: Chromosome O/Idh

3.51 Inversion - enzyme allele - combinations: Chromosome O/Pgm

3.52 Inversion - enzyme allele - combinations: Chromosome U/Mdh-2

3.53 Inversion - enzyme allele - combinations: Chromosome U/Me

3.54 Inversion - enzyme allele - combinations: Chromosome U/Idh

3.55 Inversion - enzyme allele - combinations: Chromosome U/Pgm

3.56 Inversion - enzyme allele - combinations: Chromosome E/Mdh-2

3.57 Inversion - enzyme allele - combinations: Chromosome E/Me

3.58 Inversion - enzyme allele - combinations: Chromosome E/Idh

3.59 Inversion - enzyme allele - combinations: E/Pgm

3.60 Inversion - enzyme allele - combinations: Chromosome O/Me: R. Initial

3.61 Inversion - enzyme allele - combinations: Chromosome O/Me: I_4

3.62 Inversion - enzyme allele - combinations: Chromosome O/Me: II_3

3.63 Inversion - enzyme allele - combinations: Chromosome O/Me: I_4 and II_3

4.1 Number of allozymes found for each enzyme loci

4.2 Allele frequencies of allozyme loci in European populations

4.3 Significant changes in allele frequencies: Cage I and IV

4.4 Significant changes in allele frequencies: Cage II and III

4.5 Frequencies of heterozygotes per chromosome

4.6 Gene arrangement frequencies in Europe - Chromosome I

4.7 Gene arrangement frequencies in Europe - Chromosome O

4.8 Gene arrangement frequencies in Europe - Chromosome U

4.9 Gene arrangement frequencies in Europe - Chromosome E

4.10 Gene arrangement frequencies in Europe - Chromosome A

4.11 Degree of chromosomal heterozygosity in Europe - individual chromosomes.

4.12 Total degree of chromosomal heterozygosity - Europe

4.13 Comparison of enzyme allele frequencies for larval and adult analysis of the same sample

CHAPTER ONE

INTRODUCTION

1.1 Genetic Polymorphism

The phenomenon of genetic polymorphism, that is the maintenance within a population of at least two genetically different forms, the rarest of which cannot be explained by recurrent mutation, has become increasingly important for evolutionary theory in recent years. Controversy concerning the role of natural selection versus random processess as the factors maintaining genic variability of enzyme loci is far from being settled despite the large amounts of data available concerning genic variability within natural and laboratory populations. With chromosomal structural arrangements, on the other hand, selection has been determined to be the primary factor controlling polymorphic systems. By further investigations of these sources of variation, enzymes as the primary gene product and inversions altering the structural arrangements of the genes, insight into the role of selection versus random factors in the process of evolution is hoped to be obtained.

1.2 Enzyme Polymorphism

According to Beadles one-gene-one-enzyme hypothesis, the enzyme is considered to be the primary product of the gene with any changes in its composition being directly attributable to changes in the DNA of the gene itself. Using this concept, along with the development of the 'zymogram' technique by Hunter and Markert (1957), Lewontin and Hubby opened a new way for the area of population genetics in 1966 through the application of gel electrophoresis to detect the hidden enzyme variability present within natural populations. Since

this now considered 'classic' work, much effort has been put into determining the amount of genic variability on which selection or random forces may work. This had led to the confirmation of the original results of Lewontin and Hubby: namely the median proportion of polymorphic loci in lower animals is about 30 percent and the median heterozygosity per individual is around 10.6 percent (Lewontin, 1974: pg. 118).

Furthermore, this electrophoretic separation of enzymes has been used as a taxonomic and phylogenetic tool (for studies using Drosophila see: Ayala et al, 1970; Hubby and Throckmorton, 1968; Lakovaara et al, 1971; and Nair et al, 1971).

The behavior of enzyme polymorphism with respect to environmental parameters has also been investigated. Allozyme variation for several Drosophila species was found to be remarkably similar throughout the range of the species, despite differences in the relative marginality or centrality of the local populations (Ayala et al, 1972; Saura et al, 1973; and Prakash, 1973). Even in isolated populations of Drosophila willistoni where a sharp reduction is seen in the inversion polymorphism, allozyme variation remained constant (Ayala, Powell, and Dobzhansky, 1971).

1.3 Chromosomal polymorphism

Natural populations of many Drosophila species exhibit chromosomal polymorphism which evolved and was maintained in the populations, presumably by the superior fitness of heterokaryotypes. Suppression of recombination in heterokaryotypic individuals allows selection to build up and protect coadapted gene complexes within or near the inverted regions. Results from experimental populations established with gene arrangements of similar geographic origin compared to those

from geographically separated populations support this view (Dobzhansky, 1948; and Dobzhansky and Pavlovsky, 1953). Positive position effects have also been considered important since it was realized that breakage points in naturally occurring inversions are not randomly distributed (Bernstein and Goldschmidt, 1961; and Kunze-Muhl and Sperlich, 1955) and may even coincide with those of X-ray induced inversions (Sperlich, 1959).

1.4 Stable versus Flexible Chromosomal Polymorphism

In 1962, Dobzhansky divided the chromosomal polymorphism seen in natural populations of Drosophila into two extreme types. Based on the behavior of the polymorphic system to changes in the environment, one could classify a species as having either a 'stable' or a 'flexible' structural polymorphism. 'Flexible' was used to describe those species which responded to a change in the environment by a corresponding change in their gene arrangement proportions. On the other hand, a species which maintained a particular structural polymorphism composition despite environmental changes would be classified as exhibiting a 'rigid' polymorphism.

Drosophila pseudoobscura was used as a good example of a species with a 'flexible' chromosomal polymorphism. Both natural and laboratory populations have been observed to undergo changes in the relative frequencies of the various karyotypes depending on temperature (Pavlovsky and Dobzhansky, 1966; Dobzhansky, 1970; and references therein). Likewise, seasonal cyclic changes and differences attributable to altitudinal changes within a single locality or between geographically very close localities were observed in nature.

Drosophila pavani, on the other hand, did not show large differences in structural type frequencies in geographically close

yet ecologically quite different populations (Brncic, 1957, 1961). Even over large geographic areas, this uniformity was maintained. Thus, Drosophila pavani would be classified as having a 'rigid' polymorphism.

Similarly, Dobzhansky referred to the structural polymorphism seen in Drosophila subobscura as 'rigid', drawing on results from Kunze-Muhl, Mueller and Sperlich (1958), and Stumm-Zollinger (1953) which showed no seasonal change throughout the year in the natural populations of their local area. Since then, work by Pentzos-Daponte (1964), Prevosti (1964), and Kunze-Muhl and Sperlich (1962, 1963) have supported this assumption of stability.

On the other hand, Burla and Gotz (1965) have observed seasonal fluctuations in Zurich, as has Krimbas (1964) in Greek populations. This the latter author attributed to D. subobscura responding to changes in temperature.

As far as widely separated geographic locations are concerned, there are definitely significant differences in the structural composition of the local populations (Sperlich, 1961). Thus assuredly, D. subobscura is not an example of an extreme case of 'rigid' chromosomal polymorphism.

Furthermore, analysis of data from throughout Europe (Prevosti, 1964, 1972; Krimbas, 1964; and Sperlich, 1964) implies a relationship between the amount of polymorphism and the position of the population within the species range. Marginal populations showed reduced levels of chromosomal polymorphism with central populations having high levels of variability. This was not the case with the enzyme polymorphism. (Saura et al., 1973).

1.5 Purpose of this study

Although intriguing differences in behavior has been observed for genic and chromosomal variability, investigations attempting to interrelate the two are rare. Ideally, such work would incorporate studying a natural population exhibiting both types of polymorphism and then investigating laboratory populations under defined conditions.

The important questions to be concerned with in such a study are:

What is the level of genic and chromosomal polymorphism in a natural population of D. subobscura ?

Are these polymorphic systems stable through time when brought into the laboratory?

How do they respond to a change in temperature?

Are there associations among or between the polymorphic systems present in nature?

Do such associations develop in the laboratory; are they stable?

CHAPTER TWO

MATERIAL AND METHODS

2.1 Study organism

The Drosophila obscura group proper (Sturtevant, 1942) consists of a number of European, American, and a few Asian and African species. Drosophila subobscura, a member of this group, is a widely distributed European North African species with a highly developed inversion polymorphism (Jungen, 1968) and considerable genic variability. It extends from its marginal northern European populations in Finland throughout Europe, North Africa and Asia Minor.

2.2 Trapping of Drosophila

Bait: Drosophila bait was prepared by adding three parts (by weight) of ground barley to four parts boiling water and simmering for 30 minutes. While still hot, 400 mls. were poured into two-liter plastic buckets and covered immediately to prevent bacterial and mold infections. When cool, each bucket was innoculated with a fresh yeast solution, covered, and allowed to ferment at room temperature for one week before used.

Trapping: Starting at the beginning of March, six bait buckets were placed at ten meter intervals at each of two sites (250 meters apart) in a mixed deciduous forest located 1.4 kilometers from the laboratory. A list of typical plants making up the flora of these two areas is seen in Table 2.1. These bait buckets were visited each late afternoon, weather permitting, throughout March, April and May. The flies present were collected with the aid of a nylon net cover placed over the tub thereby trapping the flies. A siphoning

TABLE 2.1. List of representative plants at collection sites in a mixed deciduous forest (Schoenbuch) in Tuebingen, West Germany.

	Site 1	Site 2
Forest Canopy	Pinus silvestris-D Fagus silvatica-F Acer pseudo-platanum-S	Fraxinus excelsior-F Fagus silvatica-F Acer pseudo-platanum-F Betula sp.-S
Lower Canopy	Coryllus avellana-D Carpinus betulus-S	Fraxinus excelsior Acer pseudo-platanum Crataegus oxyacantha
Bush Level	Crataegus oxyacantha Rhamnus fangula	
Ground Cover	Carex bricoides Ficana verum Fragaria vesca Rosa sp. Violoa sp.	Anemone nemorosa Asperula odorata Bromus sp. Carex bricoides Carex silvatica Dactylis glomerata Geum urganum Hedera helix Milium effusum Poa trivialis Primula elatior Ranunculus aunicomus Stellana holostea Vicia sepium Viola sp.

D-dominant
F-frequent
S-seldom

(The help of the Lehrstuhl für Botanik and in particular Herr G. Kost are gratefully acknowledged.)

exhauster mechanism was inserted through a hole in this cover and the flies were sucked into a vial (refer to diagram 2.1). The bait was replaced every two weeks eliminating the possibility of catching flies hatched from the bait itself.

2.3 Maintenance of flies

The flies caught were sorted as to species using Shorrocks (1972) morphological key. Drosophila subobscura females were placed individually in round plastic vials (8cm. x 3.5 cm; 61 ml.) containing fresh medium. This medium, identical to that for the cage populations, was prepared using the following receipe:

1 liter water

8 grams agar

10 grams dry yeast

80 grams sugar

63 grams grits

40 grams malt (ground barley)

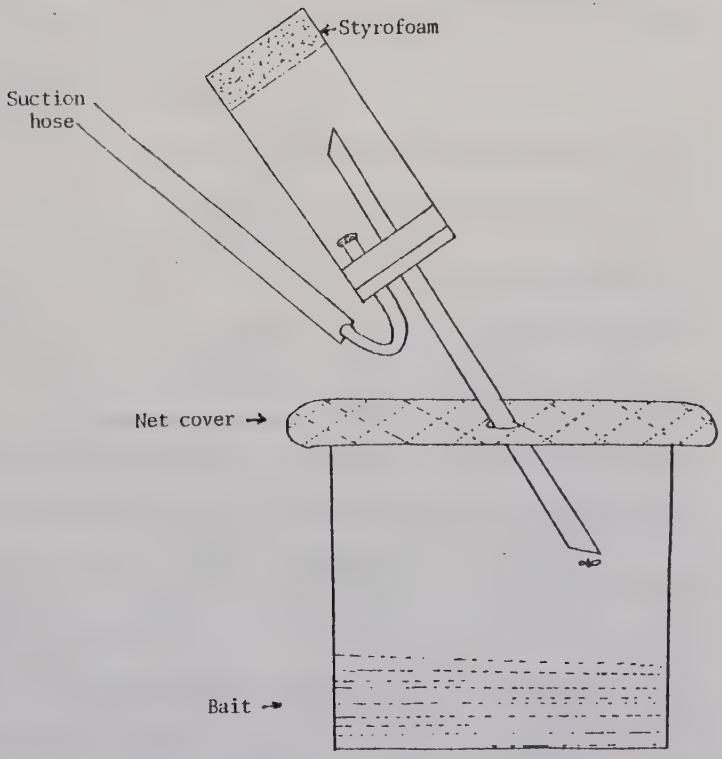
63 grams rolled oats

4 grams Napagin (Methyl 4-hydroxy-benzoate) dissolved in alcohol.

Fresh yeast solution

The first six ingredients were combined, brought to a boil, and allowed to simmer for 15 minutes. The oats were then added and cooked an additional 15 minutes. The medium was allowed to cool slightly before the napagin-alcohol mixture as a mold inhibitor was added. Small vials were then filled with approximately 10 ml. of warm medium. When cool, each vial was innoculated with a fresh yeast solution and a piece of absorbant paper was embedded in the

DIAGRAM 2.1. Siphoning exhauster.



medium. For the cage populations the medium was put into large glass bottles. Foam rubber stoppers were used to close the vials.

2.4 Establishment and Maintenance of Cage Populations

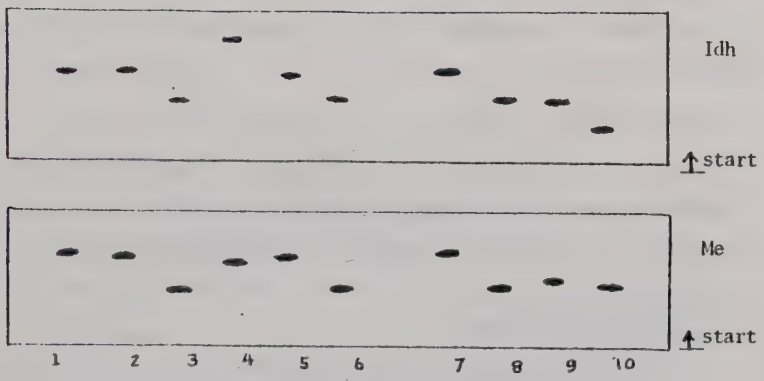
The females caught from nature were kept in their individual vials for two weeks following capture insuring adequate offspring numbers. They were then electrophoresed for five enzyme assays:

	Abbreviation	Located on chromosome
α Glycero-phosphate dehydrogenase-3	α Gpdh-3	U
Malate dehydrogenase-2	Mdh-2	U
Malic enzyme	Me	O
Isocitrate dehydrogenase	Idh	I
Phosphoglucomutase	Pgm	I

(See Lukas and Krimbas, 1975; and Lankinen and Pinsker, 1977.) The morphological species determination was rechecked at this point by use of electrophoresis data from Lakovaara and co-workers (1972). See Diagram 2.2 for schematic representation of species confirmation gel.

Offspring from the 416 D. subobscura females were then used to establish four identical cage populations to be used in this study. All vials were emptied of adult flies one week before extracting the individuals for the cage populations, thus insuring that all founders chosen were approximately the same age. Four different experimental populations were established June 10, 1976 by placing two males and two females from each vial (1664 flies) in each of the four wooden populations cages (50 cm. x 30 cm. x 10 cm.) with side screens and a glass top. Only 6 bottles containing medium were originally present in a cage. Each week two fresh medium bottles were added to the cage until the maximum of fifteen bottles were present. Thereafter, every week the two oldest

DIAGRAM 2.2. Drosophila species determination by starch electrophoresis. (After Lakovaara et al, 1972.)



Results

	Me	Idh	Species	
1	1.06 x 1.06	1.05 x 1.05	D. subobscura	
2	1.06 x 1.06	1.05 x 1.05	D. subobscura	
3	1.00 x 1.00	1.00 x 1.00	D. obscura	
4.	1.05 x 1.05	1.11 x 1.11	D. helvetica	
5	1.06 x 1.06	1.05 x 1.05	D. subobscura	
6	1.00 x 1.00	1.00 x 1.00	D. obscura	
7	1.06 x 1.06	1.05 x 1.05	D. subobscura	Controls
8	1.00 x 1.00	1.00 x 1.00	D. obscura	
9	1.01 x 1.01	1.00 x 1.00	D. ambigua	
10	1.003x 1.003	.95 x ..95	D. bifasciata	

Ten individual specimens were electrophoresed. Idh was stained in slice one, Me in slice two. Listing of the alleles for both loci yielded the species. Actual species determination was made by staining all five enzyme loci-αGpdh, Mdh, Me, Pgm and Idh

bottles were replaced by new ones thereby maintaining a fresh supply of medium. This procedure was repeated three times resulting in four identical cage populations of known initial enzyme allele frequency. The cages were kept at a uniform temperature of 18 degrees C. for two months. With this procedure, it was therefore possible to establish duplicate cage populations consisting of the genotypes found in nature at close to their natural frequencies. This is important since several experiments (Levene, Pavlovsky, and Dobzhansky, 1958; Anderson et al, 1968; and Watanabe et al, 1970) have indicated that the fitness of the karyotypes may be dependent on the composition of the genepool and may also be frequency dependent.

2.5 Sampling Procedure

The first egg sample from the experimental populations was taken from cage I after two months (August 10, 1976) by placing six new bottles in the cage (retaining the six old to be put back at the end of the sampling time). After one week, the new bottles were removed, replaced by the old ones, and set aside in the same 18 degree environment until the offspring hatched. Samples from the other three cages followed similarly at one week intervals. After the first sample was taken from cage I and cage IV, these populations were transferred to 15 degrees C. for the rest of the study period. Samples were then taken at intervals corresponding to every 3-4 generations.

The allele frequencies for the five enzyme systems were determined by electrophoresis of 300 individuals taken randomly from the 6 sample bottles.

2.6 Chromosome Analysis

From sample one of cages I and II (Initial I_1 and II_1) and samples I_4 and II_3 at the end of the study, males were crossed to females from the Kuesnacht strain known to be homozygous for the most common allele for all five enzyme systems and for the standard configuration for all five rod shaped chromosomes. Kuesnacht has also been analyzed with respect to enzyme alleles by Lankinen and Pinsker, 1977. The strain is homozygous for the enzyme loci studied. The vials containing the larvae from these crosses were transferred to gradually cooler temperatures with a constant supply of fresh yeast, thus maximising larval growth. From the progeny of each cross, the salivary glands of one larvae were dissected out in 7% saline solution and placed in a drop of filtered orcein solution. This solution was prepared by boiling five grams of orcein in 250 ml. of 60% acetic acid for two hours using a condensing coil cooling system. After staining for 5-10 minutes, the salivary glands were pressed between a previously cleaned slide smeared with albumin and a cover slip in a drop of 60% acetic acid containing trace amounts of orcein. The slides were left standing overnight in 96% alcohol and were sealed in euporal the following day. All chromosome preparations were read at the end of the study using the chromosome map of Kunze-Muhl and Miller (1958).

The remaining part of each dissected larvae was frozen immediately and electrophoresed the following day for four enzyme systems (α Gpdh-3 does not stain in the larvae).

2.7 Enzyme analysis

The starch gel electrophoresis technique used was taken after Ayala's method (Ayala et al, 1972; and Shaw and Prasad, 1970) with

some modifications. Buffer solutions: The various buffer solutions used were made according to the following recipes.

Electrode Bridge Buffer pH 7.1 s.t.p.

163.5 grams Tris. (hydroxymethyl) aminomethan $C_4H_{11}NO_3$

90.3 grams Citric acid $C_6H_8O_7 \cdot H_2O$

10 liters distilled water

Gel Buffer

267 mls. electrode bridge buffer made up to 1 liter with distilled water

Tris-HCl staining buffers

8.5 pH 20 grams Tris (hydroxymethyl) aminomethan made up to 1 liter with distilled water

7.5 pH 15 grams Tris (hydroxymethyl) aminomethan made up to 1 liter with distilled water

Buffers are titrated to desired pH with 1 N HCl

Starch Gels: Starch gels were made in two different sizes using hydrolysed Sigma starch, gel buffer and distilled water.

	Small Gel	Large Gel
Plexiglas mold	1 x 15 x 18 (cm.)	1 x 18x26 (cm.)
Starch	47 grams	73 grams
Gel buffer	90 ml.	140 ml.
Distilled water	270 ml.	420 ml.

The starch was first dissolved in a erylenmeyer flask (1l.) containing the buffer solution at room temperature. The mixture must be vigorously and continuously swirled. Boiling distilled water was added intermittently swirling between each addition to ensure uniform gel consistancy. The flask was then heated shortly over a bunsen burner and evacuated for approximately twenty seconds.

The mixture was then poured immediately into the plexiglas mold, allowed to cool to room temperature on an even horizontal surface, and covered with plastic wrap to prevent drying. All gels were prepared the day before the electrophoresis took place.

Sample preparation: Single flies were frozen in small individual wells in a plastic block the night previous to the electrophoresis. On the following day, the block was removed from the freezer, one drop of electrode buffer added to each well and the flies ground using an electric drill.

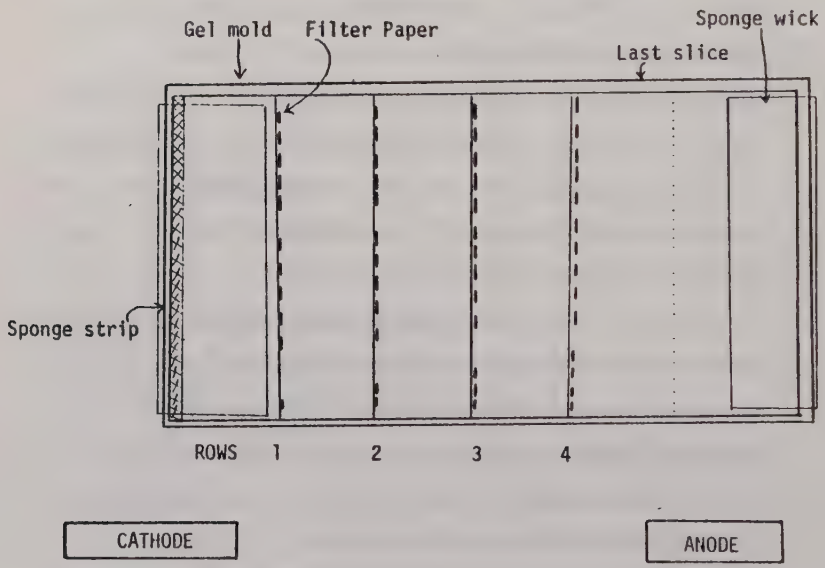
Strips of filter paper (4mm. x 11mm.) were used to absorb the crude homogenate. These strips were then inserted in a slot cut in the gel 6.5 cm. (4.5 for large gels) from the cathodal end. When using large gels, three more slots at 4.5 cm. intervals were made allowing four rows of paper strips to be run simultaneously. (See diagram 2.3 for apparatus set up.)

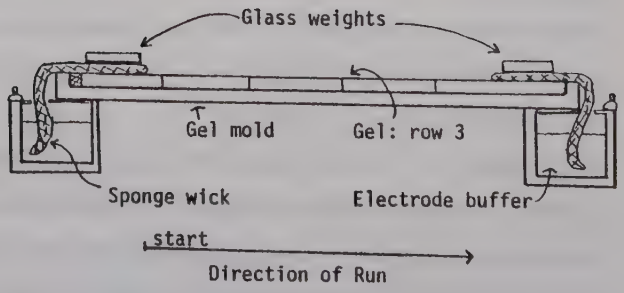
A small strip of sponge cut to fit along the cathodal end of the gel was dampened with electrode bridge buffer and inserted to insure continuous contact throughout the length of the gel. The gel was then laid horizontally over the two electrode buffer tanks, contact being made with the gel surface by sponge wicks extending into the buffer solution.

Electrophoresis: The gels ran for five hours with 40 m.A. in an enzyme laboratory at 4 degrees C, care being taken to keep the voltage lower than 200. At the end of the run, the gel was cut using the 6 stringed apparatus seen in figure 2.4 such that seven equal layers resulted. Of these, the top and bottom were discarded, leaving the remaining five to be placed with the aid of a plastic strip in individual staining tanks (10 x 20.5 x 4 cm.). The

DIAGRAM 2.3

Arrangement of gels containing sample extracts for starch electrophoresis





appropriate staining solution is then added to each tank.

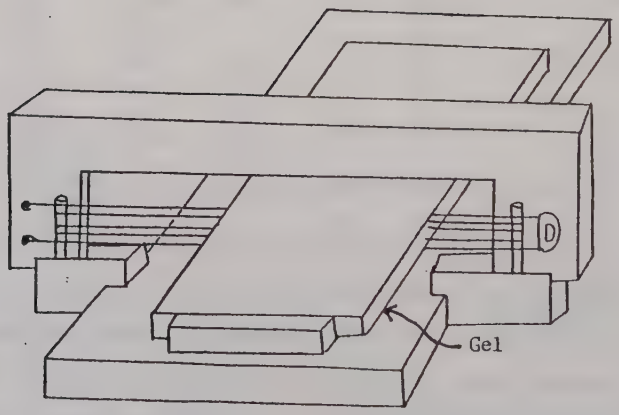
Staining: The staining solutions were made up according to the following directions:

	P.M.S.	N.B.T.	N.A.D.	N.A.D.P.	$MgCl_2$	Misc.	Substrate	Buffer
α Gpdh-3	5	25	25	-	-	-	400 Glycerophophate	8.5
Mdh-2	5	25	25	-	-	-	50 Malic acid	8.5
Me	5	20	-	20	100	-	50 Malic acid	8.5
Idh	5	20	-	15	20	-	100 Isocitrate	7.5
Pgm	5	20	25	15	250	50 EDTA 100 unit Glu-6-pdh	600 Glu-1 phosphate	7.5

All measurements are milligrams unless otherwise specified. The dry chemicals were weighed out into individual beakers, one for each enzyme system. For the buffer solution, 100 ml. was added and the solution placed on a magnetic stirrer until dissolved. With Pgm, Glucose-6-phosphodehydrogenase was added just before staining the gel slice. Each solution received 5 mg. of PMS immediately before it was poured onto the gel. For a list of chemicals used and full names for the abbreviated forms see Table 2.2. A list of the apparatus used is given in Table 2.3.

The gels were then incubated at 37 degrees C. The results were read the same day and recorded. Gels were placed in a fixing solution of 50 parts ethanol; 15 parts acetic acid; 35 parts distilled water overnight. The following day they were sealed in plastic.

DIAGRAM 2.4. Gel cutting apparatus and foil used to separate thin layers for the various enzyme staining.



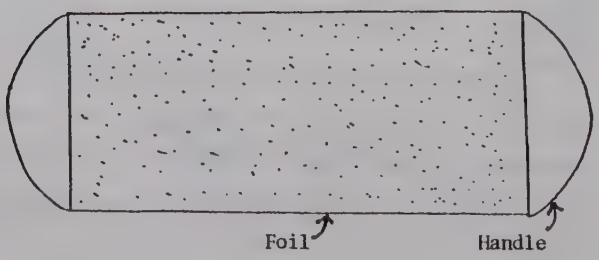


TABLE 2.2. List of chemicals.

	Abbreviation	Distributor
Acetic Acid		Merck
Aceton		Merck
Citric Acid (anhydrous)		Merck
Ethanol 95% (methylated)		Merck
Ethylenediamine Tetraacetic Acid (Disodium salt)	EDTA	Merck
Euporal		
Glucose-1-phosphate (Na_2 salt)	Glu-1-p	Boehringer
Glucose-6-phophate-dehydrogenase	G-6pdh	Boehringer
DL α-Glycerophosphate	α Gp	Serva
Hydrocloric Acid	HCl	Merck
DL Isocitric Acid (Na_3salt.$2H_2O$)		Serva
Magnesium Chloride	$MgCl_2$	Merck
Methanol 96%		Merck
B Nicotinamide Adenine Dinucleotide (grade I)	B NAD	Boehringer
B Nicotinamide Adenine Dinucleotide Phosphate Na_2 salt	NADP	Boehringer
Nitro Blue Tetrazolium	NBT	Serva
Orcein		Serva
Phenazine Methosulfate	PMS	Serva
Sigma Hydrolyzed Starch		Sigma
Tris (hydroxymethyl) aminomethane	Tris	Merck

TABLE 2.3. List of Apparati used for study.

Preparation of fly samples

Deep freezer -20° C.

Refrigerator 4° C.

Borsch electric drill

Schott Mainz cold light lamp

Plexiglas slabs with wells

Electrophoresis

Voltage generator: Labor Mueller Hann-Muenden
Desaga Heidelberg

Gel slicer

Gel trays

Staining tanks

Incubator Heraeus

pH meter WTW DIGI 510

Magnetic hot plate IKA Combimag RCH

Scale: Sauter

Precision scale: Mettler H 32

Water vacuum pump

Borsch plastic sealer

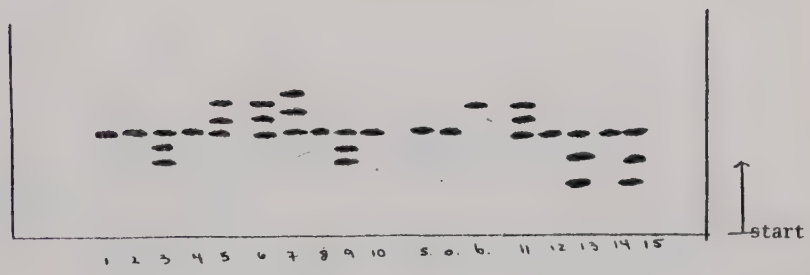
Chromosome analysis

Binocular Miscroscope: Zeiss

Phase Contrast Microscope: Zeiss

Camera: Leica M2

DIAGRAM 2.5. Allele determination for ∝Gpdh-3

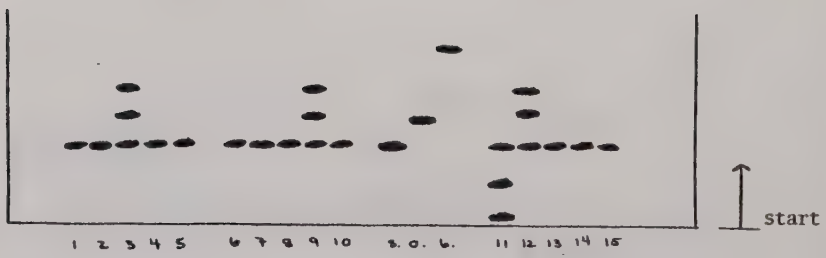


1.00 x 1.00	1,2,5,8,10,12,14
1.00 x 1.05	4,6
1.00 x 1.06	7
1.00 x 1.04	11
.91 x 1.00	13,15
.95 x 1.00	9

Controls

s	D. subobscura	1.00 x 1.00
o	D. obscura	1.00 x 1.00
b	D. bifasciata	1.04 x 1.04

DIAGRAM 2.6. Allele determination for Mdh-2

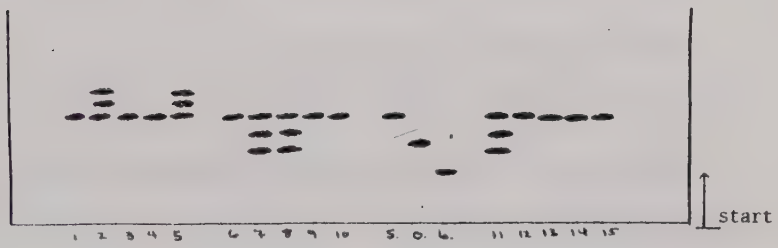


.96 x .96	1,2,4,5,6,7,8,10,13,14,15
.96 x 1.05	3,9,12
.89 x .96	11

Controls

s	D. subobscura	.96 x .96
o	D. obscura	1.00 x 1.00
b	D. bifasciata	1.11 x 1.11

DIAGRAM 2.7. Allele determination for Idh.

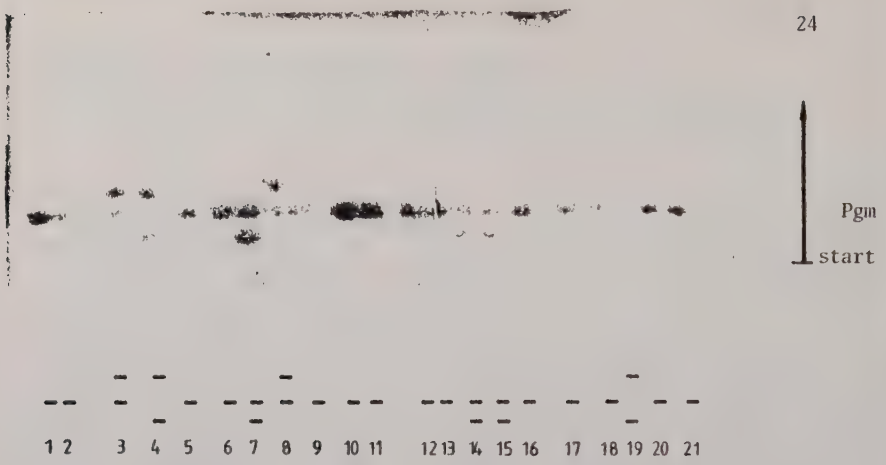


1.05 x 1.05	1,3,4,6,9,10,12,13,14,15
1.05 x 1.09	2,5
.95 x .95	7,8,11

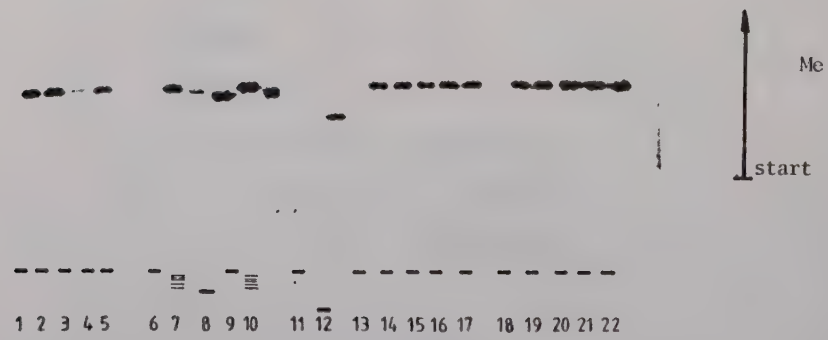
Controls

s	D. subobscura	1.05 x 1.05
o	D. obscura	1.00 x 1.00
b	D. bifasciata	.95 x .95

DIAGRAM 2.8. Allele determination for Me and Pgm



1.01 x 1.01	1,2,5,6,9,10,11,12,13,16,17,18,20,21
1.01 x 1.03	3,8
.97 x 1.01	7,]4,]5
.97 x 1.03	4,]9



1.06 x 1.06	1,2,3,4,5,6,9,13 through 22		
1.05 x 1.06	7,10		
1.05 x 1.05	8		
Controls:	11	D. subobscura	1.06 x 1.06
	12	D. obscura	1.00 x 1.00

Designation of Enzyme System Alleles: The allele designation follows the system originally by Lakovaara and Saura (1971) and adapted for D. subobscura by Lankinen and Pinsker (1977) whereby the most common allele of Drosophila obscura in every enzyme system is specified as 1.00. Alleles running faster in a gel than this control are designated by higher numbers (i.e., a five-unit faster running allele would be 1.05), those running slower by lower numbers.

By using a variety of control flies of other species kept in the laboratory or mutant subobscura laboratory stocks fixed for a particular allele, the alleles of the original flies from nature could be determined. The F_1 of all females from nature which carried rare alleles were then run simultaneously within the same gel to allow designation of the 'slow' and 'fast' alleles of each enzyme system. Sample gels allele determination are schematically represented in diagrams 2.5 for α Gpdh-3, 2.6 for Mdh-2, and 2.7 for Idh. Actual photographs of Me and Pgm are seen in Diagram 2.8.

CHAPTER THREE

Results

3.1 Enzyme Analysis

Enzyme Analysis of Natural Population

A list of alleles for αglycerophosphate dehydrogenase (α Gpdh), malate dehydrogenase (Mdh), malic enzyme (Me), isocitrate dehydrogenase (Idh), and Phosphoglucomutase (Pgm) found in the original sample of 416 Drosophila subobscura females in nature is given in table 3.1. Table 3.2 shows the original allele frequencies along with percent heterozygotes, for these adult founders of the laboratory cage populations.

Periodic Enzyme Analysis of Cage Populations

Tables 3.3 to 3.6 show the frequencies for the most common (+), slow (s), and fast (f) alleles in the original and all subsequent adult samples from each of the cage populations. Populations are referred to as Cage I, Cage II, Cage III, and Cage IV. Comparisons of allele frequencies to those of the preceeding sample within the same cage yielded chi square values shown in tables 3.7 to 3.11. In all chi square tests, data from the rare classes were pooled only when necessary to avoid more than 20% of the expected values falling below 5. Even with pooling, there were some cases where this prerequisite was not satisfied. In these cases the distribution of normal probabilities tests was used (see appendix I for exact formula used).

3.2 Chromosomal Analysis of Cage I and Cage II (larval samples)

All chromosomal arrangements found are listed below. The absolute and relative frequenices of gene arrangements in the two cages are given in tables 3.12 to 3.17 together with Chi square comparisons in tables 3.18 and 3.19. See Appendix II for summary of changes

Chromosomal arrangements found in the population of Drosophila subobscura from Tuebingen, West Germany.

Chromosome	A	I	O	U	E
	A st	I st	O st	U st	E st
	A 1	I 1	O 3+4	U 1+2	E 1+2
	A 2		O 3+4+8	U 1+2+6	E 1+2+9
			O 6	U 1+2+8	E 8
			O 3+4+8+2	U 1	E 1+2+9+12
			O 3+4+2		
			O 3+4+6		
Total = 22	3	2	7	5	5

st = standard configuration

3.3 Association Analysis

Associations between Enzyme Alleles

The larval samples used for chromosomal analysis (initial combined I_1 and II_1; final I_4 and II_3) were also analyzed electrophoretically. Therefore, enzyme allele information is not only available through periodic sampling of adults from the various population cages (as recorded in tables 3.3 through 3.6) but also for the initial and final larval samples from cage I and cage II.

The absolute and relative frequencies for Mdh-2, Me, Idh, and Pgm alleles for the initial (I_1 and II_2 combined) and final larval samples (I_4 and II_3) are given in table 3.19 and 3.20. For all analyses involving allele associations, the reduced initial sample (R. initial) is used since the complete initial sample (C. initial) includes 25 individuals for which no enzyme information is available.

For calculations concerned only with karyotype frequencies, the larger complete initial sample is used. The chi square values

for comparisons of allele frequencies between larval samples are given in table 3.21.

Absolute and relative frequencies for individuals heterozygous for alleles in the various enzyme systems are shown in table 3.22. The observed and expected numbers of individuals heterozygous for alleles in 0, 1, 2, 3, or 4 enzyme systems are listed in table 3.23. Chi square values are included.

Observed and expected values of genotypic combinations of enzyme alleles for the combined larval data are shown in tables 3.24 through 3.29. Interchromosomal and intrachromosomal comparisons are indicated. Those for Mdh/Pgm association in the individual larval samples are given in tables 3.30 through 3.32 with data for R. Initial and Cage I being pooled in table 3.33. The chi square values are included in each table.

Observed and expected values for associations between Mdh and Pgm allele combinations in the original adult sample and the final adult samples in Cage I (I_4) are seen in tables 34 and 35. The corresponding chi square values are included.

Associations between Chromosomal Arrangements

Table 3.36 gives the absolute and relative frequencies of individuals heterokaryotypic for inversions of autosomes. The observed and expected numbers of individuals heterozygous for inversions in 0, 1, 2, 3, or 4 autosomes are shown in table 3.3. Chi square values are included.

Observed and expected values for combinations of the various chromosomal arrangements for the combined larval data are seen in tables 3.38 through 3.43 along with the corresponding chi square values. Inter and intra chromosomal combinations are indicated.

Association Between Chromosomal Arrangements and Enzyme Alleles

Observed and expected values for combinations of chromosomal arrangements and enzyme alleles for the combined larval data are shown in the following tables:

Chromosome I	Tables	3.44	to	3.47
Chromosome O	Tables	3.48	to	3.51
Chromosome U	Tables	3.52	to	3.55
Chromosome E	Tables	3.56	to	3.59

Intrachromosomal and interchromosomal combinations are indicated depending on which chromosome the enzyme loci lie.

Associations between chromosome O arrangements and Me alleles for the individual samples are seen in tables 3.60 to 3.62 with data for Cage I and Cage II combined in table 3.63. Chi square values are included in each table.

Reminder: as noted in materials and methods all larvae come from crosses between sampled individuals from the populations cages and flies of the Kuesnacht laboratory strain known to be homozygous for the " plus" allele in all five studied enzyme loci and the standard chromosome arrangement for all chromosomes.

TABLE 3.1. Alleles found for five enzyme systems in the original adult samples from nature (N=416).

	α Gpdh-3	Mdh-2	Me	Idh	Pgm
Slow alleles	.91*	.89	1.01*	.99	.95
	.95*		1.05		.97
					.98
					.99
Most common allele	1.00	.96	1.06	1.05	1.01
Fast alleles	1.04*	1.05	1.07	1.09	1.02*
	1.05*				1.03
	1.06				1.05
					1.09
Total Number of Alleles	6	3	4	3	9

N = number of individuals

* = allele was found only once

Most common of the slow and fast alleles is underlined.

Alleles are designated by a number which refers to their electrophoretic mobility as compared to the most common allele of that enzyme system in Drosophila obscura (arbitrarily denoted by 1.00).

TABLE 3.2. Original frequency of enzyme alleles of Drosophila subobscura adult females from a natural population in Tuebingen, West Germany.

	Alleles			
Enzyme System	+	s	f	H
α Gpdh-3	.9916	.0024	.0060	.0167
Mdh-2	.9760	.0084	.0156	.0471
Me	.9339	.0493	.0168	.1251
Idh	.9724	.0144	.0132	.0541
Pgm	.9411	.0240	.0349	.1125

+ = most common allele

s = slow allele

f = fast allele

TABLE 3.3. Relative frequencies of enzyme alleles for Cage I (15°C) adult samples.

		Sample			
	generation	3	7	9	12
Allele	original	I_1	I_2	I_3	I_4
α Gpdh-3					
+	.9916	1.00	.9860*	.9983	.9984
s	.0024		.0109	.0017	.0016
f	.0060		.0031		
H	.0167	.0000	.0277	.0034	.0032
N	(416)	(333)	(320)	(298)	(308)
Mdh-2					
+	.9760	.9817	.9938	.9974	.9919
s	.0084	.0117	.0031	.0026	.0032
f	.0156	.0066	.0031		.0049
H	.0471	.0361	.0123	.0052	.0161
N	(416)	(300)	(320)	(388)	(308)
Me					
+	.9339	.9484	.9286*	.9246	.9039
s	.0493	.0287	.0604	.0741	.0880
f	.0168	.0229	.0110	.0013	.0081
H	.1251	.0992	.1339	.1396	.1752
N	(416)	(349)	(273)	(398)	(307)
Idh					
+	.9724	No data	.9759	.9742	.9805
s	.0144		.0086	.0014	
f	.0132		.0155	.0244	.0195
H	.0541		.0473	.0503	.0382
N	(416)		(291)	(349)	(308)
Pgm					
+	.9411	.9534*	.9639	.9510	.9204*
s	.0240	.0354	.0330	.0465	.0325
f	.0349	.0122	.0031	.0025	.0471
H	.1125	.0896	.0698	.0934	.1496
N	(416)	(268)	(318)	(398)	(308)
Ave. H	.0711	.0562	.0582	.0584	.0765

* = significant difference from allele frequencies of previous sample.

H = heterozygosity

N = number of individuals = 1/2 number of alleles

TABLE 3.4. Relative frequencies of enzyme alleles for Cage II (18°C) adult samples.

		Sample			
	generation	3	10	12	15
Allele	original	II_1	II_2	II_3	II_4
α Gpdh-3					
+	.9916	1.00*	1.00	.9983	1.00
s	.0024			.0017	
f	.0060				
H	.0167	0	0	.0034	0
N	(416)	(492)	(296)	(296)	(269)
Mdh-2					
+	.9760	.9755*	.9781	.9712	.9793
s	.0084	.0225	.0219	.0220	.0169
f	.0156	.0020		.0068	.0038
H	.0471	.0479	.0428	.0562	.0407
N	(416)	(510)	(297)	(295)	(266)
Me					
+	.9339	.9190	.8451*	.9139*	.8825*
s	.0493	.0482	.0842	.0642	.1045
f	.0168	.0328	.0707	.0219	.0130
H	.1251	.1521	.2737	.1602	.2101
N	(416)	(488)	(297)	(296)	(268)
Idh					
+	.9724	No data	.9812	.9695	.9551
s	.0144		.0188	.0271	.0449
f	.0132			.0034	
H	.0541		.0369	.0593	.0858
N	(416)		(293)	(295)	(267)
Pgm					
+	.9411	.9140	.8771*	.8699	.8717
s	.0240	.0334	.0741	.0531	.0688
f	.0349	.0526	.0488	.0770	.0595
H	.1125	.1607	.2228	.2345	.2319
N	(416)	(494)	(297)	(292)	(269)
Ave. H	.0711	.0902	.1153	.1027	.1137

* = significant difference from allele frequencies of previous sample.

H = heterozygosity

N = number of individuals = 1/2 number of alleles

TABLE 3.5. Relative frequencies of enzyme alleles for Cage III (18°C) adult samples.

		Sample			
	Generation	3	7	10	12
Allele	original	III_1	III_2	III_3	III_4
α Gph-3					
+	.9916	1.00	.9850*	1.00*	.9950
s	.0024		.0123		.0050
f	.0060		.0027		
H	.0167	0	.0295	0	.0100
N	(416)	(325)	(365)	(298)	(300)
Mdh-2					
+	.9760	.9905	.9948	1.00	.9717*
s	.0084	.0095	.0017		.0283
f	.0156		.0035		
H	.0471	.0189	.0104	0	.0550
N	(416)	(315)	(285)	(298)	(300)
Me					
+	.9339	.9730*	.9535	.9144*	.9682*
s	.0493	.0270	.0349	.0856	.0318
f	.0168		.0116		
H	.1251	.0525	.0895	.1565	.0616
N	(416)	(371)	(344)	(298)	(299)
Idh					
+	.9724	.9853	No data	.9710	.9900*
s	.0144	.0092		.0119	.0100
f	.0132	.0055		.0171	
H	.0541	.0291		.0567	.0198
N	(416)	(271)		(293)	(299)
Pgm					
+	.9411	.9536*	.9546	.9111*	.9650*
s	.0240	.0082	.0109	.0252	.0050
f	.0349	.0382	.0345	.0637	.0300
H	.1125	.0891	.0874	.1652	.0679
N	(416)	(366)	(507)	(298)	(300)
Ave. H	.0711	.0379	.0542	.0757	.0428

* = significant difference from allele frequencies in previous samples.

H = heterozygosity

N = number of individuals = 1/2 number of alleles

TABLE 3.6. Relative frequencies of enzyme alleles for Cage IV (15°C) adult samples.

		Sample			
	generation	3	8	10	13
Allele	original	IV_1	IV_2	IV_3	IV_4
α Gpdh-3					
+	.9916	.9985	1.00	1.00	1.00
s	.0024	.0015			
f	.0060				
H	.0167	.0030	0	0	0
N	(416)	(333)	(294)	(296)	(296)
Mdh-2					
+	.9760	.9792	.9596	.9747*	.9645
s	.0084	.0055	.0185	.0206	.0220
f	.0156	.0153	.0219	.0047	.0135
H	.0471	.0409	.0783	.0495	.0691
N	(416)	(360)	(297)	(316)	(296)
Me					
+	.9339	.9567*	.9799*	.9597	.9763
s	.0493	.0144	.0072	.0177	.0169
f	.0168	.0289	.0129	.0226	.0068
H	.1251	.0837	.0396	.0782	.0465
N	(416)	(416)	(348)	(310)	(296)
Idh					
+	.9724	.9630	.9934	.9860	.9831
s	.0144	.0185	.0033	.0140	.0152
f	.0132	.0185	.0033		.0017
H	.0541	.0719	.0131	.0276	.0333
N	(416)	(54)	(298)	(393)	(296)
Pgm					
+	.9411	.9578*	.9484	.9057*	.9442*
s	.0240	.0341	.0502	.0440	.0507
f	.0349	.0081	.0014	.0503	.0051
H	.1125	.0814	.0980	.1752	.1059
N	(416)	(308)	(349)	(318)	(296)
Ave. H	.0711	.0562	.0458	.0661	.0510

* = significant difference from allele frequencies in previous sample.

H = heterozygosity

N = number of individuals = 1/2 number of alleles

TABLE 3.7. Chi square values for comparisons of α Gpdh-3 allele frequencies between adult samples within the same cage population as determined by normal distribution of probability.

	Comparison between samples	N	Data Pooled	Z value	P value	d.f.	Significance
Cage I	Original/I_1	749	s and f	1.68	.095	1	N.S.
	I_1/I_2	653	s and f	2.17	.0300	1	Sign. (3%)
	I_2/I_3	618	s and f	1.71	.0872	1	N.S.
	I_3/I_4	606	s and f	.13	.9760	1	N.S.
Cage II	Original/II_1	908	s and f	2.04	.0414	1	Sign. (4%)
	II_1/II_2	788	None	0	1.000	1	N.S.
	II_2/II_3	592	None	.71	.4778	1	N.S.
	II_3/II_4	565	None	.71	.4778	1	N.S.
Cage III	Original/III_1	741	s and f	1.66	.0970	1	N.S.
	III_1/III_2	690	s and f	2.22	.0264	1	Sign. (2.6%)
	III_2/III_3	663	s and f	2.22	.0264	1	Sign. (2.6%)
	III_3/III_4	598	None	1.22	.2224	1	N.S.
Cage IV	Original/IV_1	749	s and f	1.29	.1970	1	N.S.
	IV_1/IV_2	627	s and f	.66	.5092	1	N.S.
	IV_2/IV_3	590	None	0	1.000	1	N.S.
	IV_3/IV_4	592	None	0	1.000	1	N.S.

s and f = slow and fast alleles N.S. = not significant N = number of genes sampled

TABLE 3.8. Chi square values for comparisons of Me allele frequencies between adult samples within the same cage population.

	Comparison between samples	N	Contingency Table used	Data Pooled	X^2	d.f.	Significance level
Cage I	Original/I_1	1530	2 x 3	None	4.8545	2	N.S.
	I_1/I_2	1244	2 x 3	None	9.8608	2	.01
	I_2/I_3	1342	2 x 2	s and f	.0274	1	N.S.
	I_3/I_4	1410	2 x 2	s and f	1.6660	1	N.S.
Cage II	Original/II_1	1808	2 x 3	None	4.6150	2	N.S.
	II_1/II_2	1570	2 x 3	None	21.2851	2	.001
	II_2/II_3	1186	2 x 3	None	18.3822	2	.001
	II_3/II_4	1128	2 x 3	None	7.0442	2	.05
Cage III	Original/III_1	1574	2 x 2	s and f	12.3998	1	.001
	III_1/III_2	1430	2 x 2	s and f	3.3586	1	N.S.
	III_2/III_3	1284	2 x 2	s and f	7.4248	1	.01
	III_3/III_4	1194	2 x 2	None	14.6941	1	.001
Cage IV	Original/IV_1	1664	2 x 3	None	18.7290	2	.001
	IV_1/IV_2	1528	2 x 3	None	6.4398	2	.05
	IV_2/IV_3	1316	2 x 3	None	4.8913	2	N.S.
	IV_3/IV_4	1212	2 x 3	None	5.2055	2	N.S.

s and f = fast and slow alleles N.S. = not significant N = number of genes sampled

TABLE 3.9. Chi square values for comparisons of Mdh allele frequencies between adult samples within the same cage population.

	Comparison between samples	N	Contingency Table used	Data Pooled	X^2 or Z	d.f.	Significance level
Cage I	Original/I_1	1432	2 x 3	None	2.7455	2	N.S.
	I_1/I_2	1240	P. distrib.	s and f	1.3797	1	N.S.
	I_2/I_3	1416	P. distrib.	s and f	.7348	1	N.S.
	I_3/I_4	1392	P. distrib.	s and f	1.0183	1	N.S.
Cage II	Original/II_1	1852	2 x 3	None	16.2158	2	.001
	II_1/II_2	1614	2 x 2	s and f	.0273	1	N.S.
	II_2/II_3	1184	2 x 2	s and f	.3210	1	N.S.
	II_3/II_4	1122	2 x 2	s and f	.4635	1	N.S.
Cage III	Original/III_1	731	P. distrib.	s and f	1.4702	1	N.S.
	III_1/III_2	600	P. distrib.	s and f	.6114	1	N.S.
	III_2/III_3	583	P. distrib.	s and f	1.2464	1	N.S.
	III_3/III_4	598	P. distrib.	s and f	2.9249	1	.0036
Cage IV	Original/IV_1	1552	2 x 3	None	.4519	2	N.S.
	IV_1/IV_2	1314	2 x 3	None	5.6976	2	N.S.
	IV_2/IV_3	1226	2 x 3	None	7.0298	2	.05
	IV_3/IV_4	1224	2 x 3	None	2.6744	2	N.S.

s and f = fast and slow alleles N.S. = not significant N = number of genes sampled

TABLE 3.10. Chi square values for comparisons of Idh allele frequencies between adult samples within the same cage population.

	Comparison between samples	N	Contingency Table used	Data Pooled	X^2 or Z	d.f.	Significance level
Cage I	Original/I_2	1414	2 x 3	None	1.0951	2	N.S.
	I_2/I_3	1280	2 x 2	s and f	.0003	1	N.S.
	I_3/I_4	1314	2 x 2	s and f	.3350	1	N.S.
Cage II	Original/II_2	1417	2 x 2	s and f	.5898	1	N.S.
	II_2/II_3	1176	2 x 2	s and f	1.2313	1	N.S.
	II_3/II_4	1124	2 x 2	s and f	1.2473	1	N.S.
Cage III	Original/III_1	1374	2 x 2	s and f	1.9208	1	N.S.
	III_1/III_3	1128	2 x 2	s and f	2.0217	1	N.S.
	III_3/III_4	1184	2 x 2	s and f	4.6436	1	.05
Cage IV	Original/IV_1	940	P. distrib.	s and f	.3894	1	N.S.
	IV_1/IV_2	704	P. distrib.	s and f	1.9477	1	N.S.
	IV_2/IV_3	1382	2 x 2	s and f	1.0652	1	N.S.
	IV_3/IV_4	1378	2 x 2	s and f	.0451	1	N.S.

s and f = slow and fast alleles N.S. = not significant N = number of alleles

TABLE 3.11. Chi square values for comparisons of Pgm allele frequencies between adult samples within the same cage population.

	Comparison between samples	N	Contingency Table used	Data Pooled	X^2	d.f.	Significance level
Cage I	Original/I_1	1368	2 x 3	None	8.6739	2	.05
	I_1/I_2	1172	2 x 2	s and f	.5682	1	N.S.
	I_2/I_3	1432	2 x 2	s and f	1.1125	1	N.S.
	I_3/I_4	1412	2 x 3	None	33.4496	2	.001
Cage II	Original/II_1	1820	2 x 3	None	4.9252	2	N.S.
	II_1/II_2	1582	2 x 3	None	13.2739	2	.01
	II_2/II_3	1178	2 x 3	None	5.7926	2	N.S.
	II_3/II_4	1122	2 x 3	None	2.3991	2	N.S.
Cage III	Original/III_1	1564	2 x 3	None	6.0654	2	.05
	III_1/III_2	1746	2 x 3	None	.3428	2	N.S.
	III_2/III_3	1610	2 x 3	None	11.4509	2	.01
	III_3/III_4	1196	2 x 3	None	16.2847	2	.001
Cage IV	Original/IV_1	1448	2 x 3	None	12.1445	2	.01
	IV_1/IV_2	1314	2 x 2	s and f	.4474	1	N.S.
	IV_2/IV_3	1334	2 x 3	None	33.0630	2	.001
	IV_3/IV_4	1228	2 x 3	None	22.8049	2	.001

s and f = slow and fast alleles N.S. = not significant N = number of alleles

TABLE 3.12. Absolute and relative frequencies of chromosomal structural types for Chromosome A among larval samples of Cage I and Cage II. (Only female larvae are considered here.)

Larvae come from crosses with Kuesnacht laboratory stain (see materials and methods, or page 29 of text).

		CHROMOSOME A (X chromosome)			
SAMPLE	N	A st./st.	A 1/st.	A 2/st.	
Complete Initial	52	36.0	14.0	2.0	abs. freq.
$I_1 + II_1$		.6923	.2692	.0385	rel. freq.
Reduced Initial	39.5	29.5	10.0	0.0	abs. freq.
$I_1 + II_1$		.7468	.2532		rel. freq.
Cage I_4	75	60.0	10.0	5.0	abs. freq.
Final		.80	.1333	.0667	rel. freq.
Cage II_3	150	105.0	37.0	8.0	abs. freq.
Final		.70	.2467	.0533	rel. freq.

See Appendix II for graphic summary of major changes in chromosomal arrangement frequencies.

TABLE 3.13. Absolute and relative frequencies of chromosomal structural types for Chromosome I among larval samples of Cage I and Cage II.

		CHROMOSOME I		
SAMPLE	N	I st./st.	I 1/st.	
Complete Initial $I_1 + II_1$	104	75.0	29.0	abs. freq.
		.7212	.2788	rel. freq.
Reduced Initial $I_1 + II_1$	79	57.0	22.0	abs. freq.
		.7215	.2785	rel. freq.
Cage I_4 Final	150	68.0	82.0	abs. freq.
		.4533	.5467	rel. freq.
Cage II_3 Final	300	222.0	78.0	abs. freq.
		.74	.26	rel. freq.

See Appendix II for graphic summary of major changes in chromosomal arrangement frequencies.

TABLE 3.14. Absolute and relative frequencies of Chromosome O structural types among larval samples of Cage I and Cage II.

		CHROMOSOME O							
SAMPLE	N	O st./st.	O 3+4/st.	O 3+4+8/st.	O 6/st.	O 3+4+8+2/st.	O 3+4+2/st.	O 3+4+6/st.	
Complete Initial	104	73.0	15.0	11.0	4.0	0.0	1.0	0.0	abs. freq.
$I_1 + II_1$		.7019	.1442	.1058	.0385		.0096		rel. freq.
Reduced Initial	79	54.0	12.0	10.0	3.0	0.0	0.0	0.0	abs. freq.
$I_1 + II_1$		.6835	.1519	.1266	.0380				rel. freq.
Cage I_4	150	105.0	5.0	35.0	4.0	0.0	0.0	1.0	abs. freq.
Final		.70	.0333	.2333	.0267			.0067	rel. freq.
Cage II_3	300	184.0	47.0	33.0	29.0	1.0	5.0	1.0	abs. freq.
Final		.6133	.1567	.11	.0967	.0033	.0167	.0033	rel. freq.

See Appendix II for graphic summary of major changes in chromosomal arrangement frequencies.

TABLE 3.15. Absolute and relative frequencies of Chromosome U structural types among larval samples of Cage I and Cage II.

		CHROMOSOME U					
SAMPLE	N	U st./st.	U 1+2/st.	U 1+2+6/st.	U 1+2+8/st.	U 1/st.	
Complete Initial	104	80.0	16.0	4.0	3.0	1.0	abs. freq.
$I_1 + II_1$		.7692	.1539	.0385	.0288	.0096	rel. freq.
Reduced Initial	79	63.0	12.0	2.0	1.0	1.0	abs. freq.
$I_1 + II_1$		.7974	.1519	.0253	.0127	.0127	rel. freq.
Cage I_4	150	104.0	30.0	2.0	12.0	2.0	abs. freq.
Final		.6933	.20	.0133	.08	.0133	rel. freq.
Cage II_3	300	250.0	34.0	10.0	6.0	0.0	abs. freq.
Final		.8333	.1133	.0333	.02		rel. freq.

See Appendix II for graphic summary of major changes in chromosomal arrangement frequencies.

TABLE 3.16. Absolute and relative frequencies of Chromosome E structural types among larval samples of Cage I and Cage II.

		CHROMOSOME E					
SAMPLE	N	E st./st.	E 1+2/st.	E 1+2+9/st.	E 8/st.	E 1+2+9+12/st.	
Complete Initial	104	93.0	5.0	1.0	5.0	0.0	abs. freq.
$I_1 + II_1$		.8942	.0481	.0096	.0481		rel. freq.
Reduced Initial	79	71.0	4.0	1.0	3.0	0.0	abs. freq.
$I_1 + II_1$		.8987	.0506	.0127	.0380		rel. freq.
Cage I_4	150	137.0	7.0	5.0	0.0	1.0	abs. freq.
Final		.9133	.0467	.0333		.0067	rel. freq.
Cage II_3	300	250.0	22.0	11.0	16.0	1.0	abs. freq.
Final		.8333	.0733	.0367	.0533	.0033	rel. freq.

See Appendix II for graphic summary of major changes in chromosomal arrangement frequencies.

TABLE 3.17. Chi square values for comparisons between larval samples of Cage I and Cage II for A, I, and E chromosome arrangement frequencies.

	Comparison between samples	Compared	N	Contingency Table used	Data Pooled	X^2	d.f.	Significance level
Chromosome A	C.In./Cage I_4	Cage I Initial & Final	127	2 x 2	A1,A2	1.3907	1	None
	C.In./Cage II_3	Cage II Initial & Final	202	2 x 3	None	.2537	2	None
	Cage I_4/Cage II_3	Finals of Cages I & II	225	2 x 3	None	3.9101	2	None
Chromosome I	C.In./Cage I_4	Cage I Initial & Final	254	2 x 2	None	16.8335	1	.001
	C.In./Cage II_3	Cage II Initial & Final	404	2 x 2	None	.0607	1	None
	Cage I_4/Cage II_3	Finals of Cages I & II	450	2 x 2	None	34.6241	1	.001
Chromosome E	C.In./Cage I_4	Cage I Initial & Final	254	2 x 2	E1+2 E8,E1+2+9, E1+2+9+12	.4343	1	None
	C.In./Cage II_3	Cage II Initial & Final	404	2 x 4	E1+2+9, E1+2+9+12	3.3312	3	None
	Cage I_4/Cage II_3	Finals of Cages I & II	450	2 x 4	E8 E1+2+9+12	8.1289	3	.05

C.In. = complete initial sample N = number of chromosomes

TABLE 3.18. Chi square values for comparisons between larval samples of Cage I and Cage II for O and U chromosomal arrangement frequencies.

	Comparison between samples	Compared	N	Contingency Table used	Data Pooled	X^2	d.f.	Significance level
Chromosome O	C.In./Cage I	Cage I Initial & Final	254	2 x 4	06,03+4+2, O 3+4+6	15.4506	3	.01
	C.In./Cage II	Cage II Initial & Final	404	2 x 5	03+4+2, 03+4+8+2, 03+4+6	4.9802	4	None
	Cage I/Cage II	Finals of Cage I & II	450	2 x 5	03+4+2, 03+4+8+2, 03+4+6	32.6435	4	.001
Chromosome U	C.In./Cage I	Cage I Initial & Final	254	2 x 3	U1,U1+2+6, U1+2+8	1.7858	2	None
	C.In./Cage II	Cage II Initial & Final	404	2 x 3	U1,U1+2+6, U1+2+8	2.1361	2	None
	Cage I/Cage II	Finals of Cages I & II	450	2 x 3	U1,U1+2+6, U1+2+8	11.7728	2	.01

C.In. = Complete initial sample N = number of chromosomes

TABLE 3.19. Absolute and relative frequencies of enzyme alleles for larval data from Cage I and Cage II.

Sample	N	Mdh-2 + (.96)	Mdh-2 s (.89)	Mdh-2 f (1.05)	Me + (1.06)	Me s (1.05)	Me f (1.07)	allele designation
R. Initial	79.0	70.0	1.0	8.0	69.0	8.0	2.0	abs. freq.
$I_1 + II_1$		.8861	.0127	.1012	.8734	.1013	.0253	rel. freq.
	.2044				.2263			H
Cage I_4	150.0	138.0	0.0	12,0	139.0	10.0	1.0	abs. freq.
		.92		.08	.9267	.0667	.0066	rel. freq.
	.1472				.1367			H
Cage II_3	300.0	287.0	3.0	10.0	259.0	31.0	10.0	abs. freq.
		.9567	.0100	.0333	.8633	.1033	.0333	rel. freq.
	.0835				.2429			H

H = heterozygosity

R. Initial = reduced initial sample

TABLE 3.20. Absolute and relative frequencies of enzyme alleles for larval data from Cage I and Cage II.

Sample	N	Idh + (1.05)	Idh s (.99)	Idh f (1.09)	Pgm + (1.01)	Pgm s (.97)	Pgm f (1.05)	allele designation
R. Initial	79.0	79.0	0.0	0.0	64.0	8.0	7.0	abs. freq.
$I_1 + II_1$		1.00			.8101	.1013	.0086	rel. freq.
	0.0				.3256			H
Cage I_4	150.0	147.0	0.0	3.0	129.0	20.0	1.0	abs. freq.
		.98		.02	.86	.1333	.0067	rel. freq.
	.0392				.2426			H
Cage II_3	300.0	288.0	11.0	1.0	267.0	14.0	19.0	abs. freq.
		.96	.0367	.0033	.89	.0467	.0633	rel. freq.
	.0770				.2017			H

H = heterozygosity

R. Initial = reduced initial sample

TABLE 3.21. Chi square values for comparisons between larval samples from Cage I and Cage II for enzyme system allele frequencies.

	Comparison between samples	N	Contingency Table used	Data Pooled	X^2 or Z	d.f.	Significance level
Mdh-2	R.In./Cage I_4	229	P. distrib.	s and f	.8450	1	N.S.
	R.In./Cage II_3	379	P. distrib.	s and f	2.39	1	.016
	Cage I_4/Cage II_3	450	2 x 2	s and f	1.9112	1	N.S.
	R.In./I_4/II_3	529	3 x 2	s and f	6.0423	2	.05
Me	R.In./Cage I_4	229	2 x 2	s and f	2.3235	1	N.S.
	R.In./Cage II_3	379	2 x 3	None	.1369	2	N.S.
	Cage I_4/Cage II_3	450	2 x 3	None	4.8382	2	N.S.
Idh	R.In./Cage I_4	229	P. distrib.	s and f	1.2653	1	N.S.
	R.In./Cage II_3	379	P. distrib.	s and f	1.8065	1	N.S.
	Cage I_4/Cage II_3	450	P. distrib.	s and f	1.1142	1	N.S.
Pgm	R.In./Cage I_4	229	2 x 3	None	10.5335	2	.01
	R.In./Cage II_3	379	2 x 3	None	4.2505	2	N.S.
	Cage I_4/Cage II_3	450	2 x 3	None	17.2684	2	.001
	R.In./I_4/II_3	529	3 x 3	None	19.3601	4	.001

N = number of chromosomes R.In. = reduced initial sample s and f = slow and fast alleles

TABLE 3.22. Absolute and relative frequencies of individuals heterozygous for alleles of enzyme systems in larval samples of Cage I and Cage II.

Sample	N	Mdh-2	Me	Idh	Pgm	
Reduced Initial $I_1 + II_1$	79	9.0	10.0	0.0	15.0	abs. freq.
		.1139	.1266		.1899	rel. freq.
Cage I_4	150	12.0	11.0	3.0	21.0	abs. freq.
		.08	.0733	.02	.14	rel. freq.
Cage II_3	300	13.0	41.0	12.0	33.0	abs. freq.
		.0433	.1367	.04	.11	rel. freq.

TABLE 3.23. Observed and expected numbers of individuals heterozygous for enzyme allele in 0, 1, 2, 3 or 4 enzyme systems in larval samples of Cage I and Cage II.

		Individuals heterozygous for alleles in the following number of enzyme loci.						
Sample	N	0	1	2	3 or 4		X^2	d.f.
Reduced Initial $I_1 + II_1$	79	55.0	14.0	10*	0*	observed	13.0443++	2
		49.5305	25.1553	4.0979*	.2163*	expected		
Cage I_4	150	110.0	33.0	7*	0*	observed	1.9018	2
		107.7773	37.6457	4.3792*	.1978*	expected		
Cage II_3	300	212.0	77.0	11*	0*	observed	.0764	2
		211.7004	78.0877	9.7313*	.4806*	expected		

* data pooled for calculation of chi square

++ significant to the .01 level

TABLE 3.24. Allele combinations between enzyme systems for total of larval data (N = 529) from Cage I and Cage II.

	Interchromosomal (U and O)			
Mdh-2 (Chromosome U)	+/+	s/+	f/+	allele
Me (Chromosome O)	(.9357)	(.0076)	(.0567)	(rel. freq.)
+/+	435.0	4*	28.0	observed
(.8828)	436.9730	3.5492	26.4790	expected
s/+	48.0	0*	1*	observed
(.0926)	45.8356	.3723	2.7775	expected
f/+	12.0	0*	1*	observed
(.0246)	12.1766	.0989	.7379	expected

N = number of alleles studied X^2 = .5140 d.f. = 4 * = data from these classes pooled for X^2

TABLE 3.25. Allele combinations between enzyme systems for total of larval data (N = 529) from Cage I and Cage II.

	Interchromosomal (U and I)			
Mdh-2 (Chromosome U)	+/+	s/+	f/+	allele
Idh (Chromosome I)	(.9357)	(.0076)	(.0567)	(rel. freq.)
+/+	489.0	3*	22.0	observed
(.9716)	480.9277	3.9062	29.1425	expected
s/+	11.0	0*	0*	observed
(.0208)	10.2957	.0836	.6239	expected
f/+	4*	0*	0*	observed
(.0076)	3.7619	.0306	.2280	expected

N = number of alleles studied $X^2 = 2.2435$ d.f. = 4 * = data pooled for chi square test.

TABLE 3.26. Allele combination between enzyme systems for total of larval data (N = 529) from Cage I and Cage II.

	Interchromosomal (I and O)			
Idh (Chromosome I)	+/+	s/+	f/+	allele
Me (Chromosome O)	(.9716)	(.0208)	(.0076)	(rel. freq.)
+/+	465.0	8.0	4*	observed
(.8828)	453.7384	9.7136	3.5492	expected
s/+	38.0	3*	0*	observed
(.0926)	47.5942	1.0189	.3723	expected
f/+	11.0	0*	0*	observed
(.0246)	12.6438	.2707	.0989	expected

N = number of alleles studied $X^2 = 3.2674$ d.f. = 4 * = data pooled for chi square test.

TABLE 3.27. Allele combinations between enzyme systems for total of larval data (N = 529) from Cage I and Cage II.

	Intrachromosomal - chromosome I			
Idh (Chromosome I)	+/+	s/+	f/+	allele
Pgm (Chromosome I)	(.9716)	(.0208)	(.0076)	(rel. freq.)
+/+	460.0	11.0	4*	observed
(.8696)	446.9539	9.5684	3.4961	expected
s/+	34.0	0*	0*	observed
(.0794)	40.8097	.8737	.3192	expected
f/+	20.0	0*	0*	observed
(.0510)	26.2128	.5612	.2050	expected

N = number of alleles studied $X^2 = 3.5920$ d.f. = 4 * = data from these classes pooled for chi square test.

TABLE 3.28. Allele associations between enzyme systems for total larval data (N = 529) from Cage I and Cage II.

	Interchromosomal (I and U)			
Pgm (Chromosome I)	+/+	s/+	f/+	allele
Mdh-2 (Chromosome U)	(.8696)	(.0794)	(.0510)	(rel. freq.)
+/+	441.0	31.0	23.0	observed
(.9357)	430.4392	39.3018	25.2443	expected
s/+	2*	2*	0*	observed
(.0076)	3.4961	.3192	.2050	expected
f/+	18.0	8.0	4*	observed
(.0567)	26.0830	2.3815	1.5297	expected

N = number of individuals sampled $\chi^2 = 19.0540$ d.f. = 5 sign. .01 level

* = data pooled for chi square test.

TABLE 3.29. Allele association between enzyme systems for total of larval data (N = 529) from Cage I and Cage II.

	Interchromosomal (I and O)			
Pgm (Chromosome I)	+/+	s/+	f/+	allele
Me (Chromosome O)	(.8696)	(.0794)	(.0510)	(rel. freq.)
+/+	407.0	37.0	24.0	observed
(.8828)	406.1042	37.0799	23.8171	expected
s/+	43.0	4*	1*	observed
(.0926)	42.5977	3.8894	2.4983	expected
f/+	11.0	1*	1*	observed
(.0246)	11.3165	1.0333	.6637	expected

N = number of individuals sampled X^2 = .1617 d.f. = 5 * = data pooled for chi square test.

TABLE 3.30. Allele associations between Mdh and Pgm for individual larval samples Reduced Initial (N = 79) $\overline{I_1 \text{ and } II_1}$.

	Interchromosomal (U and I)			
Mdh-2 (Chromosome U)	+/+	s/+	f/+	allele
Pgm (Chromosome I)	(.8861)	(.0127)	(.1012)	(rel. freq.)
+/+	61.0	.0*	3.0	observed
(.8101)	56.7085	.8128	6.4766	expected
s/+	5.0	1**	2**	observed
(.1013)	7.0912	.1016	.8099	expected
f/+	4*	0*	3**	observed
(.0886)	6.2022	.0889	.7083	expected

X^2 = 16.0086 d.f. = 4 sign. .01 level * = data pooled for chi square test.
** = data pooled for chi square test.

TABLE 3.31. Allele associations between Mdh and Pgm for individual larval samples Cage I_4 (N = 150).

	Interchromosomal (U and I)			
Mdh-2 (Chromosome U)	+/+	s/+	f/+	allele
Pgm (Chromosome I)	(.92)	(0)	(.08)	(rel. freq.)
+/+	123.0		6.0	observed
(.86)	118.6800		10.32	expected
s/+	14.0		6*	observed
(.1333)	18.3954		1.5996	expected
f/+	1*		0*	observed
(.0067)	.9246		.0804	expected

X^2 = 10.4333 d.f. = 3 sign. .05 level * = data pooled for chi square test.

TABLE 3.32. Allele associations between Mdh and Pgm for larval sample Cage II_3 (N = 300).

	Interchromosomal (U and I)			
Mdh-2 (Chromosome U)	+/+	s/+	f/+	allele
Pgm (Chromosome I)	(.9567)	(.01)	(.0333)	(rel. freq.)
+/+	256.0	2*	9.0	observed
(.89)	255.4389	2.67	8.8911	expected
s/+	13.0	1*	0*	observed
(.0467)	13.4034	.1401	.4665	expected
f/+	18.0	0*	1*	observed
(.0633)	18.1677	.1899	.6324	expected

X^2 = .0186 d.f. = 4 * = data pooled for chi square test.

TABLE 3.33. Allele associations between Mdh and Pgm for larval samples Cage I_4 and R. Initial (N = 229).

	Interchromosomal (U and I)			
Mdh-2 (Chromosome U)	+/+	s/+	f/+	allele
Pgm (Chromosome I)	(.9083)	(.0044)	(.0873)	(rel. freq.)
+/+	184.0	0*	9.0	observed
(.8428)	175.3030	.8492	16.8490	expected
s/+	19.0	1*	8*	observed
(.1223)	25.4385	.1232	2.4450	expected
f/+	5.0	0*	3*	observed
(.0349)	7.2592	.0352	.6977	expected

X^2 = 21.2672 d.f. = 4 sign. .001 level * = data pooled for chi square test.

TABLE 3.34. Mdh/Pgm genotype combinations for the original 416 adult flies from nature.

	Interchromosomal (U and I)			
Mdh-2 (Chromosome U)	+/+	+/s	+/f	
Pgm (Chromosome I)	(.9519)	(.0168)	(.0313)	(rel. freq.)
+/+	349.0	7.0	13.0	observed
(.8870)	351.2435	6.1991	11.5494	expected
+/s & s/s	19.0	0*	0*	observed
(.0457)	18.0968	.3194	.5951	expected
+/f & f/f	28.0	0*	0*	observed
(.0673)	26.6502	.4703	.8763	expected

X^2 = 2.6745 d.f. = 5 * = data pooled for chi square test. +/+ = homozygote for most common allele.

+/s and +/f = heterozygotes for most common allele with rare (slow or fast) allele.

s/s and f/f = homozygotes for rare (slow or fast) alleles.

TABLE 3.35. Mdh/Pgm genotype combinations for adult sample I_4 from Cage I.

Mdh-2 (Chromosome U)	Interchromosomal (U and I)			
	+/+	+/s	+/f	
Pgm (Chromosome I)	(.9838)	(.0065)	(.0097)	
+/+	258.0	2*	3*	observed
(.8539)	258.7406	1.7095	2.5511	expected
+/s	16.0	0*	0*	observed
(.0519)	15.7262	.1039	.1551	expected
+/f	25.0	0*	0*	observed
(.0812)	24.6044	.1626	.2426	expected
s/f	4*	0*	0*	observed
(.0130)	3.9391	.0262	.0388	expected

X^2 = .0138 d.f. = 3 * = data pooled for chi square test.

+/s and +/f = heterozygotes for most common allele and rare (slow or fast) allele.

+/+ = homozygotes for most common allele. s/f = heterozygote for rare (slow and fast) alleles.

TABLE 3.36. Absolute and relative frequencies of heterokaryotic individuals for inversions of autosomes.

Sample	N	Chromosome I	O	U	E	
Complete Initial (I_1 + II_1)	104	29.0	31.0	24.0	11.0	abs. freq.
		.2788	.2981	.2309	.1058	rel. freq.
Cage I_4	150	82.0	45.0	46.0	13.0	abs. freq.
		.5467	.30	.3067	.0867	rel. freq.
Cage II_3	300	78.0	116.0	50.0	50.0	abs. freq.
		.26	.3867	.1667	.1667	rel. freq.

N = number of individuals

TABLE 3.37. Observed and expected numbers of individuals heterozygous for inversions in 0, 1, 2, 3, or 4 autosomes.

Sample	N	Individuals heterozygous for inversions in the following number of chromosomes: 0	1	2	3 or 4		X^2	d.f.
Complete Initial (I_1+II_1)	104	40.0	39.0	19.0	6.0	observed	2.4608	3
		36.2124	44.5269	19.5201	3.7406	expected		
Cage I_4	150	34.0	55.0	52.0	9.0	observed	4.3028	3
		30.1424	65.4589	43.3088	11.0899	expected		
Cage II_3	300	102.0	116.0	68.0	14.0	observed	2.9282	3
		94.5556	130.6555	61.86	12.9289	expected		

N = number of chromosomes sampled

TABLE 3.38. Distribution of inversion combinations for Chromosomes I and U of larval samples (N = 554).

	U st./st. (.7834)	U 1+2/st. (.1444)	U 1+2+6/st. (.0289)	U 1+2+8/st. and U 1/st. (.0433)	(rel. freq.)
I st./st. (.6588)	293.0	49.0	12.0	11.0	observed
	285.9216	52.7024	10.5478	15.8034	expected
I 1/st. (.3412)	141.0	31.0	4.0	13.0	observed
	148.0820	27.2952	5.4628	8.1848	expected

$X^2 = 6.1613$ d.f. = 7 N = number of chromosomes sampled

TABLE 3.39. Distribution of inversion combinations for Chromosomes I and E of larval samples (N = 554).

	E st./st. (.8664)	E 1+2/st. (.0614)	E 1+2+9/st. and E 1+2+9+12/st. (.0343)	E 8/st. (.0379)	(rel. freq.)
I st./st.	308.0	28.0	15.0	14.0	observed
(.6588)	316.2145	22.4095	12.5186	13.8326	expected
I 1/st.	172.0	6.0	4.0	7.0	observed
(.3412)	163.7711	11.6061	6.4836	7.1640	expected

N = number of chromosomes sampled $X^2 = 6.1784$ d.f. = 7

TABLE 3.40. Distribution of combinations for Chromosomes I and O for total of larval samples (N = 554).

	O st./st. (.6534)	O 3+4/st. (.1209)	O 3+4+8/st. (.1426)	O 6/st. (.0668)	Others/st. (.0163)	(rel. freq.)
I st./st. (.6588)	244.0	38.0	49.0	28.0	6.0	observed
	238.4748	44.1255	52.0455	24.3803	5.9491	expected
I 1/st. (.3412)	118.0	29.0	30.0	9.0	3.0	observed
	123.5088	22.8531	26.9549	12.6269	3.0811	expected

N = number of chromosomes sampled $X^2 = 4.9814$ d.f. = 9

TABLE 3.41. Distribution of inversion combinations for Chromosomes U and O for total larval samples (N = 554).

	U st./st. (.7834)	U 1+2/st. (.1444)	U 1+2+6/st. (.0289)	U 1+2+8/st. and U 1/st. (.0433)	(rel. freq.)
O st./st. (.6534)	281.0	53.0	11.0	17.0	observed
	283.5780	52.2704	10.4613	15.6739	expected
O 3+4/st. (.1209)	58.0	5.0	2*	2*	observed
	52.4710	9.6717	1.9357	2.9002	expected
O 3+4+8/st. (.1426)	61.0	13.0	2**	3**	observed
	61.8889	11.4077	2.2831	3.4207	expected
O 6/st. (.0668)	26.0	8.0	1#	2#	observed
	28.9914	5.3438	1.0695	1.6024	expected
Others/st. (.0163)	8.0	1#	0#	0#	observed
	7.0743	1.3040	.2610	.3910	expected

N = number of chromosomes sampled * = data pooled for chi square X^2 = 5.3143 d.f. = 13

** = data pooled for chi square # = data pooled for chi square

TABLE 3.42. Distribution of inversion combinations for Chromosomes U and E for total of larval samples (N = 554).

	U st./st.	U 1+2/st.	U 1+2+6/st.	U 1+2+8/st. and U 1/st.	
	(.7834)	(.1444)	(.0289)	(.0433)	(rel. freq.)
E st./st.	379.0	68.0	12.0	21.0	observed
(.8664)	376.0207	69.3099	13.8716	20.7834	expected
E 1+2/st.	23.0	8*	3*	0**	observed
(.0614)	26.6478	4.9119	.9831	1.4729	expected
E 1+2+9/st. and E 1+2+9+12/st.	13.0	4#	0**	2#	observed
(.0343)	14.8863	2.7439	.5492	.8228	expected
E 8/st.	19.0	0**	1**	1**	observed
(.0379)	16.4487	3.0319	.6068	.9092	expected

N = number of chromosomes sampled * = data pooled for chi square test X^2 = 10.6969

d.f. = 9 ** = data pooled for chi square test ## = data pooled for chi square test

TABLE 3.43. Distribution of inversion combinations for Chromosomes O and E for total of larval samples (N = 554).

	O st./st. (.6534)	O 3+4/st. (.1209)	O 3+4+8/st. (.1426)	O 6/st. (.0668)	Others/st. (.0163)	(rel. freq.)
E st./st. (.8664)	316.0	54.0	72.0	31.0	7.0	observed
	313.6226	58.0303	68.4459	32.0630	7.8238	expected
E 1+2/st. (.0614)	22.0	5.0	2*	3**	2**	observed
	22.2258	4.1125	4.8506	2.2722	.5545	expected
E 1+2+9/st. and E 1+2+9+12/st. (.0343)	12.0	6.0	1*	0**	0**	observed
	12.4160	2.2974	2.7097	1.2693	.3097	expected
E 8/st. (.0379)	12.0	2#	4#	3**	0**	observed
	13.7192	2.5385	2.9941	1.4026	.3422	expected

N = number of chromosomes sampled X^2 = 10.3413 d.f. = 12 * = data pooled for chi square test

** = data pooled for chi square test # = data pooled for chi square test

TABLE 3.44. Inversion-enzyme allele combinations with Chromosome I arrangements for total of larval data N = 529. (Reduced, Initial, Cage I_4 and Cage II_3 samples)

		Interchromosomal (I and U)		
	Chromosome I	I st./st.	I 1/st.	(rel. freq.)
Mdh-2 (U)		(.6560)	(.3440)	
+/+		326.0	169.0	observed
(.9357)		324.7104	170.2749	expected
s/+		2*	2*	observed
(.0076)		2.6374	1.3830	expected
f/+		19.0	11.0	observed
(.0567)		19.6763	10.3180	expected

X^2 = .0831 d.f. = 4 * = data pooled for chi square test

TABLE 3.45. Inversion-enzyme allele combinations with Chromosome I arrangements for total of larval data N = 529.

		Interchromosomal (I and O)		
	Chromosome I	I st./st.	I 1/st.	
Me (O)		(.6560)	(.3440)	(rel. freq.)
+/+		303.0	164.0	observed
(.8828)		306.3528	160.6484	expected
s/+		32.0	17.0	observed
(.0926)		32.1344	16.8510	expected
f/+		12.0	1.0	observed
(.0246)		8.5368	4.4766	expected

$X^2 = 4.2134$ d.f. = 5

TABLE 3.46. Inversion-enzyme allele combinations with Chromosome I arrangements for total of larval data N = 529.

		Intrachromosomal - Chromosome I		
	Chromosome I	I st./st.	I 1/st.	
Idh (I)		(.6560)	(.3440)	(rel. freq.)
+/+		338.0	176.0	observed
(.9716)		337.1685	176.8079	expected
s/+		8.0	3*	observed
(.0208)		7.2181	3.7851	expected
f/+		1.0	3*	observed
(.0076)		2.6374	1.3830	expected

X^2 = 1.2409 d.f. = 4 * = data pooled for chi square test

TABLE 3.47. Inversion-enzyme allele combinations with Chromosome I arrangements for total of larval data N = 529.

		Intrachromosomal - Chromosome I		
	Chromosome I	I st./st.	I 1/st.	
Pgm (I)		(.6560)	(.3440)	(rel. freq.)
+/+		303.0	157.0	observed
(.8696)		301.7721	158.2463	expected
s/+		24.0	18.0	observed
(.0794)		27.5537	14.4489	expected
f/+		20.0	7.0	observed
(.0510)		17.6982	9.2808	expected

$X^2 = 2.2058$ d.f. = 5

TABLE 3.48. Inversion-enzyme allele combinations with Chromosome O arrangements for total of larval data N = 529.

		Interchromosomal (O and U)				
	Chromosome O	O st./st.	O 3+4/st.	O 3+4+8/st.	O 6/st.	Others/st.	
Mdh-2 (U)		(.6484)	(.1210)	(.1474)	(.0680)	(.0152)	(rel. freq.)
+/+		322.0	58.0	76.0	31.0	8.0	observed
(.9357)		320.9485	59.8932	72.9608	33.6590	7.5238	expected
s/+		4.0	0*	0*	0*	0*	observed
(.0076)		2.6068	.4865	.5926	.2734	.0611	expected
f/+		17.0	6**	2*	5**	0*	observed
(.0567)		19.4483	3.6293	4.4212	2.0396	.4559	expected

X^2 = 9.4229 d.f. = 8 * = data pooled for chi square test

** = data pooled for chi square test

TABLE 3.49. Inversion-enzyme allele combinations with Chromosome O arrangements for total of larval data N = 529.

		Intrachromosomal - Chromosome O				
Me (O)	Chromosome O	O st./st. (.6484)	O 3+4/st. (.1210)	O 3+4+8/st. (.1474)	O 6/st. (.0680)	Others/st. (.0152)	(rel. freq.)
+/+		301.0	62.0	62.0	36.0	6.0	observed
(.8828)		302.8036	56.5071	68.8360	31.7561	7.0984	expected
s/+		30.0	1.0	16.0	0.0	2*	observed
(.0926)		31.7621	5.9272	7.2204	3.3310	.7446	expected
f/+		12.0	1*	0*	0*	0*	observed
(.0246)		8.4379	1.5746	1.9182	.8849	.1978	expected

X^2 = 22.6760 d.f. = 10 * = data pooled for chi square test sign. .05 level

TABLE 3.50. Inversion-enzyme allele associations with chromosome 0 arrangements for total larval data N = 529.

		Interchromosomal (0 and I)					
	Chromosome 0	0 st./st.	0 3+4/st.	0 3+4+8/st.	0 6/st.	Others/st.	
Idh (I)		(.6484)	(.1210)	(.1474)	(.0680)	(.0152)	(rel. freq.)
+/+		333.0	63.0	74.0	36.0	8.0	observed
(.9716)		333.2623	62.1911	75.7601	34.9504	7.8124	expected
s/+		7.0	1*	3*	0*	0*	observed
(.0208)		7.1345	1.3314	1.6219	.7482	.1672	expected
f/+		3.0	0*	1*	0*	0*	observed
(.0076)		2.6068	.4865	.5926	.2734	.0611	expected

X^2 = .1646 d.f. = 7 * = data pooled for chi square test

TABLE 3.51. Inversion-enzyme allele combinations with chromosome O arrangments for total of larval data N = 529.

		Interchromosomal (O and I)					
	Chromosome O	O st./st.	O 3+4/st.	O 3+4+8/st.	O 6/st.	Others/st.	
Pgm (I)		(.6484)	(.1210)	(.1474)	(.0680)	(.0152)	(rel. freq.)
+/+		294.0	57.0	73.0	29.0	7.0	observed
(.8696)		298.2759	55.6622	67.8067	31.2813	6.9923	expected
s/+		33.0	3.0	3.0	2.0	1*	observed
(.0794)		27.2345	5.0823	6.1912	2.8562	.6384	expected
f/+		16.0	4**	2*	5**	0*	observed
(.0510)		17.4932	3.2645	3.9767	1.8346	.4101	expected

X^2 = 8.5609 d.f. = 11 * = data pooled for chi square test ** = data pooled for chi square test

hromosomal - Chromosome U

1+2/st.	U 1+2+6/st.	U 1+2+8/st. and U 1/st.	
.1437)	(.0265)	(.0415)	(rel. freq.)
.0	14.0	20.0	observed
.1294	13.1171	20.5419	expected
*	0*	0*	observed
.5777	.1065	.1668	expected
**	0**	2*	observed
.3102	.7949	1.2448	expected

r chi square test ** = data pooled for chi square test

TABLE 3.53. Inversion-enzyme allele combinations with chromosome U for total of larval data N = 529.

		Interchromosomal (U and O)				
	Chromosome U	U st./st.	U 1+2/st.	U 1+2+6/st.	U 1+2+8/st. and U 1/st.	
Me (O)		(.7883)	(.1437)	(.0265)	(.0415)	(rel. freq.)
+/+		364.0	70.0	12.0	21.0	observed
(.8828)		368.1370	67.1081	12.3755	19.3805	expected
s/+		41.0	6.0	2*	0*	observed
(.0926)		38.6152	7.0392	1.2981	2.0329	expected
f/+		12.0	0*	0*	1*	observed
(.0246)		10.2585	1.8700	.3449	.5401	expected

X^2 = 2.4789 d.f. = 7 * = data pooled for chi square test

TABLE 3.54. Inversion-enzyme allele combinations with chromosome U arrangements for total larval data N = 529.

		Interchromosomal (U and I)				
	Chromosome U	U st./st.	U 1+2/st.	U 1+2+6/st.	U 1+2+8/st. and U 1/st.	
Idh (I)		(.7883)	(.1437)	(.0265)	(.0415)	(rel. freq.)
+/+		402.0	76.0	14.0	22.0	observed
(.9716)		405.1676	73.8584	13.6204	21.3300	expected
s/+		11.0	0*	0*	0*	observed
(.0208)		8.6738	1.5812	.2916	.4566	expected
f/+		4*	0*	0*	0*	observed
(.0076)		3.1693	.5777	.1065	.1668	expected

X^2 = 1.6118 d.f. = 5 * = data pooled for chi square test

TABLE 3.55. Inversion-enzyme allele combinations with chromosome U arrangements for total larval data N = 529.

		Interchromosomal (U and I)				
	Chromosome U	U st./st.	U 1+2/st.	U 1+2+6/st.	U 1+2+8/st. and U 1/st.	
Pgm (I)		(.7883)	(.1437)	(.0265)	(.0415)	(rel. freq.)
+/+		368.0	64.0	11.0	17.0	observed
(.8696)		362.6325	66.1046	12.1905	19.0908	expected
s/+		31.0	6.0	2*	3*	observed
(.0794)		33.1106	6.0358	1.1131	1.7431	expected
f/+		18.0	6.0	1*	2*	observed
(.0510)		21.2675	3.8769	.7149	1.1196	expected

X^2 = 4.6257 d.f. = 8 * = data pooled for chi square test

TABLE 3.56. Inversion-enzyme allele combinations with chromosome E arrangements for total of larval data N = 529.

		Interchromosomal (E and U)				
	Chromosome E	E st./st.	E 1+2/st.	E 1+2+9/st. and E 1+2+9+12/st.	E 8/st.	
Mdh-2 (U)		(.8658)	(.0624)	(.0359)	(.0359)	(rel. freq.)
+/+		429.0	31.0	16.0	19.0	observed
(.9357)		428.5583	30.8871	17.7700	17.7700	expected
s/+		4*	0*	0*	0*	observed
(.0076)		3.4809	.2509	.1443	.1443	expected
f/+		25.0	2**	3**	0*	observed
(.0567)		25.9691	1.8716	1.0768	1.0768	expected

X^2 = 1.9622 d.f. = 6 * = data pooled for chi square test ** = data pooled for chi square test

TABLE 3.57. Inversion-enzyme allele combinations with chromosome E arrangements for total of larval data N = 529.

		Interchromosomal (E and O)				
	Chromosome E	E st./st.	E 1+2/st.	E 1+2+9/st. and E 1+2+9+12/st.	E 8/st.	
Me (O)		(.8658)	(.0624)	(.0359)	(.0359)	(rel. freq.)
+/+		403.0	30.0	19.0	15.0	observed
(.8828)		404.3296	29.1409	16.7653	16.7653	expected
s/+		45.0	1.0	0*	3*	observed
(.0926)		42.4116	3.0567	1.7586	1.7586	expected
f/+		10.0	2*	0*	1*	observed
(.0246)		11.2670	.8120	.4672	.4672	expected

X^2 = 2.3008 d.f. = 7 * = data pooled for chi square test

TABLE 3.58. Inversion-enzyme allele combinations with chromosome E arrangements for total of larval data N = 529.

		Interchromosomal (E and I)				
	Chromosome E	E st./st.	E 1+2/st.	E 1+2+9/st. and E 1+2+9+12/st.	E 8/st.	
Idh (I)		(.8658)	(.0624)	(.0359)	(.0359)	(rel. freq.)
+/+		443.0	33.0	19.0	19.0	observed
(.9716)		445.0008	32.0721	18.4518	18.4518	expected
s/+		11.0	0*	0*	0*	observed
(.0208)		9.5266	.6866	.3950	.3950	expected
f/+		4*	0*	0*	0*	observed
(.0076)		3.4809	.2509	.1443	.1443	expected

X^2 = .7040 d.f. = 5 * = data pooled for chi square test

TABLE 3.59. Inversion-enzyme allele combinations with chromosome E arrangements for total of larval data N = 529.

Interchromosomal (E and I)

Pgm (I)	Chromosome E	E st./st. (.8658)	E 1+2/st. (.0624)	E 1+2+9/st. and E 1+2+9+12/st. (.0359)	E 8/st. (.0359)	(rel. freq.)
+/+		403.0	23.0	18.0	16.0	observed
(.8696)		398.2839	28.7051	16.5147	16.5147	expected
s/+		34.0	6*	0**	2**	observed
(.0794)		36.3659	2.6210	1.5079	1.5079	expected
f/+		21.0	4*	1*	1**	observed
(.0510)		23.3584	1.6835	.9685	.9685	expected

X^2 = 8.1946 d.f. = 7 * = data pooled for chi square test ** = data pooled for chi square test

TABLE 3.60. Inversion-enzyme allele combinations with chromosome O arrangements and Me alleles. Reduced Initial Sample N = 79 (I_1 and II_1).

		Intrachromosomal - Chromosome O				
	Chromosome O	O st./st.	O 3+4/st.	O 3+4+8/st.	O 6/st.	
Me (O)		(.6835)	(.1519)	(.1266)	(.0380)	(rel. freq.)
+/+		44.0	12.0	10.0	3*	observed
(.8734)		47.1605	10.4809	8.7352	2.6219	expected
s/+		8.0	0*	0*	0*	observed
(.1013)		5.4698	1.2156	1.0131	.3041	expected
f/+		2.0	0*	0*	0*	observed
(.0253)		1.3661	.3036	.2530	.0760	expected

X^2 = 3.4221 d.f. = 5 * = data pooled for chi square test

TABLE 3.61. Inversion-enzyme allele combinations with chromosome O arrangements and Me alleles for Cage I_4 (N = 150).

	Intrachromosomal - Chromosome O					
Chromosome O	O st./st.	O 3+4/st.	O 3+4+8/st.	O 6/st.	Others/st.	
Me (O)	(.70)	(.0333)	(.2333)	(.0267)	(.0067)	(rel. freq.)
+/+	101.0	5.0	28.0	4*	1*	observed
(.9267)	97.3035	4.6289	32.4299	3.7114	.9313	expected
s/+	3.0	0*	7.0	0*	0*	observed
(.0667)	7.0035	.3332	2.3342	.2671	.0670	expected
f/+	1*	0*	0*	0*	0*	observed
(.0066)	.6930	.0330	.2310	.0264	.0066	expected

X^2 = 12.4044 d.f. = 5 sign. .05 level * = data pooled for chi square test

TABLE 3.62. Inversion-enzyme allele combinations with chromosome O arrangements for Me alleles for Cage II_3 (N = 300).

		Intrachromosomal - Chromosome O					
	Chromosome O	O st./st.	O 3+4/st.	O 3+4+8/st.	O 6/st.	Others/st.	
Me (O)		(.6133)	(.1567)	(.1100)	(.0967)	(.0233)	(rel. freq.)
+/+		156.0	45.0	24.0	29.0	5.0	observed
(.8633)		158.8386	40.5837	28.4889	25.0443	6.0345	expected
s/+		19.0	1.0	9.0	0*	2*	observed
(.1033)		19.0062	4.8561	3.4089	2.9967	.7221	expected
f/+		9.0	1*	0*	0*	0*	observed
(.0333)		6.1269	1.5654	1.0989	.9660	.2328	expected

X^2 = 18.3892 d.f. = 9 sign. .05 level * = data pooled for chi square test

TABLE 3.63. Inversion-enzyme associations with chromosome 0 arrangements for Cage I_4 and Cage II_3 larval data (N = 450).

		Intrachromosomal - Chromosome 0				
	Chromosome 0	0 st./st.	0 3+4/st.	0 3+4+8/st.	0 6/st.	Others/st.	
Me (0)		(.6422)	(.1156)	(.1511)	(.0733)	(.0178)	(rel. freq.)
+/+		257.0	50.0	52.0	33.0	6.0	observed
(.8845)		255.6117	46.0117	60.1416	29.1752	7.0848	expected
s/+		22.0	1.0	16.0	0*	2*	observed
(.0911)		26.3270	4.7390	6.1943	3.0049	.7297	expected
f/+		10.0	1*	0*	0*	0*	observed
(.0244)		7.0514	1.2693	1.6591	.8048	.1954	expected

X^2 = 25.3774 d.f. = 9 sign. .01 level * = data pooled for chi square test

CHAPTER FOUR

Discussion

4.1 Enzyme Analysis

4.1.1 Enzyme Polymorphism found in the natural population

Level of Polymorphism

In the electrophoresis analysis of the Tuebingen population of Drosophila subobscura, variant alleles were detected in all of the five enzyme systems examined. Only in α Gpdh-3 does the frequency of the most common allele exceed .99. Mdh-2 and Idh show moderate amounts of variation (%H around 5%), with Pgm and Me exhibiting the highest levels (%H around 11% and 13%). Pinsker, Lankinen and Sperlich (1978) reported similar levels of heterozygosity in their study of Tuebingen D. subobscura. The number of different alloyzmes detected for each system does not necessarily reflect the level of polymorphism present as illustrated below in Table 4.1.

TABLE 4.1. The number of different allozymes and heterozygosity for each of the studied enzyme systems detected among Tuebingen D. subobscura.

	α Gpdh-3	Mdh-2	Me	Idh	Pgm
Number of slow alleles:	2	1	2	1	4
Number of fast alleles:	3	1	1	1	4
Plus most common allele:	1	1	1	1	1
Equals Total:	6	3	4	3	9
Heterozygosity	.0159	.0473	.1322	.0529	.1127

Comparison to other European Localities

The most frequent variant corresponds in all enzyme systems to that established as the most common allele for the species (Saura et al, 1973) and the frequencies agree with those found in other European populations of D. subobscura as seen in Table 4.2.

α Gpdh-3, Mdh-2, and Pgm show a great deal of stability throughout Europe. Me does fluctuate somewhat with France showing an extremely high level of polymorphism while in other populations the frequency of the most common allele is well into the 90's. Tuebingen and the Southern Scandinavian populations have identical Me allele frequencies, with the more eastern localities being less variable. Idh exhibits a slightly higher degree of polymorphism in Tuebingen than is found elsewhere. Nevertheless, the initial allele frequencies of the Tuebingen population corresponds quite well to the overall situation seen throughout Europe with D. subobscura maintaining uniform allele frequencies throughout its marginal (Finland and Scandinavia) and central populations.

4.1.2 Enzyme Polymorphism in the laboratory cage populations

Tables 3.3 through 3.6 show the enzyme allele frequencies in the cage populations. All references to statistically significant changes refer to chi square values with a p value of at least .01 calculated from comparisons of one sample to the one directly preceeding it within the same cage population. Those changes showing significance at less than .01 level were attributed to sampling error (as in the case of α Gpdh-3 where three cages show fixation of the '+' allele upon establishment in the laboratory only to reveal the continued existance of the rare alleles in later samples). Such changes are indicated in Tables 4.3 and 4.4 with *.

TABLE 4.2. Allele frequencies for allozyme loci in European populations of D. subobscura.

Enzyme System	Allele	Tuebingen[1]	Finland[2]	South Scandinavia[2]	France[2]	East Alps[2]	Greece[3]
α Gpdh-3	1.00	.992	.99	.998	.99	1.00	>.99
	.95	.002	.005	---	.004	---	---
	all fast	.006	.005	.002	.01	---	---
	N	832	346	386	826	156	
	H	.016	.02	.004	.02	0	
Mdh-2	.96	.976	.96	.97	.97	.98	.99
	all slow	.008	.03	.02	.01	.02	.01
	all fast	.016	.01	.01	.02	---	---
	N	832	326	388	826	146	319
	H	.047	.077	.059	.059	.039	.02
Me	1.06	.93	.99	.93	.77	.96	>.99
	1.05	.05	.005	.06	.11	.03	---
	1.07	.02	.005	.01	.12	.01	---
	N	832	320	386	726	156	
	H	.132	.020	.131	.381	.077	
Idh	1.05	.973	.99	.99	.996	.99	
	all slow	.014	.005	.003	.004	.01	
	1.09	.013	.003	.003	---	---	
	N	832	372	388	820	158	
	H	.053	.02	.02	.008	.02	
Pgm	1.01	.941	.93	.93	.94	.94	
	all slow	.024	.03	.01	.02	.02	
	all fast	.035	.04	.06	.04	.04	
	N	832	268	386	826	156	
	H	.112	.133	.131	.114	.114	

N = number of genes sampled H = heterozygosity = $1-(p^2+q^2+r^2)$

1. present study 2. Saura et al. (1973)

3. Zouros, E. & C. Krimbas (1974)

Changes corresponding to chi square values of at least p = .01 are described.

α Gpdh-3 change from nature into laboratory and transfer from 18° to 15°

There were no significant changes in any of the cages throughout the entire experiment with allele frequencies remaining stable when the population was brought into the laboratory from nature and also when cages I and IV were transferred into the colder environment.

Final allele frequences

The small amount of variability found initially in α Gpdh-3 (H = .0167) decreased in all four of the cage populations as a result of the loss of the 'f' allele and decrease or loss of the slow allele.

Mdh-2 change from nature into the laboratory

Only cage II experienced a significant change from the initial allele frequencies, with the new values (higher "s" and lower "f") being maintained throughout the rest of the study.

Transfer from 18° to 15°

Neither cage I nor cage IV were affected by the transfer to colder temperatures.

Final allele frequencies

The original level of polymorphism (H = .0471) was consistantly maintained in cages II and IV, with cage III experiencing an initial decrease of heterozygosity due to the loss of the fast allele but recovering such that final H levels match those of the initial sample. In cage I, however, the gradual loss of the fast allele resulted in final H values being considerably lower than those initially.

Me change from nature into the laboratory

Upon establishment of the populations in the laboratory both cage III and cage IV experienced a decrease in the slow allele while the other two cages showed no changes.

Transfer from 18° to 15°

Cage I did experience an increase in the slow allele when transferred into the cold, with this high frequency being maintained throughout the rest of the study such that over 8% of the final alleles sampled from that cage were 's'. Cage IV did not show a marked response to the change but never did recover from the initial loss of the 's' allele.

Final allele frequences

The two consistantly warm cages (cage II and cage III)experienced much more fluctuation than those transferred to cold. Cage II rare allele frequencies increased in sample 2, followed by a decrease with the final analysis revealing over 10% slow allele. Cage III similarly experienced an increase in slow toward the end only to be followed by a decrease resulting in final frequencies being similar to those initially.

Idh change from nature to laboratory and transfer from 18° to 15°

There were no significant changes in the allele frequencies between subsequent samples in any of the four cages.

Final allele frequencies

Despite the loss of slow allele or fast allele in some of the cages, H values fluctuate around the initial level.

Pgm change from nature into labatory

Cage IV showed a significant response to the change with a decrease in the fast allele.

Transfer from 18° to 15°

Neither cage was significantly affected by the change to the colder environment.

Final allele frequencies

Cage I final allele frequencies match those of the initial sample. Cage II final analysis reflects the increase in the 'slow' allele experienced early in the experiment along with a gradual increase in the "fast" allele throughout the experiment.

An increase in the "slow" allele, followed by a decrease in the last two samples of Cage III resulted in the final sample consisting of only .5% "slow" compared to the original 2.4%. Cage IV, despite an increase to 5% for the "fast" allele in sample 3, shows a final value of .5% with "slow" having increased to 5%.

Summary

In general, one can say the enzyme polymorphism underwent little change when transferred to colder temperatures.

Pinsker, Lankinen and Sperlich (1978) in their recent study of D. subobscura in Tuebingen periodically analyzed the same five enzyme loci over a period of two years. No significant seasonal changes were found in nature for these loci either.

4.2 Chromosomal Analysis

4.2.1 Chromosomal polymorphism in the natural population

A highly developed inversion polymorphism is present in the Tuebingen natural population of D. subobscura. For the five acrocentric rod shaped chromosomes, 22 different structural arrangements were found. In each chromosome, the standard arrangement was the most prevalent, making up from 69% of the chromosome A arrangements to 89% of chromosome E karyotypes, with O, U and I values

TABLE 4.3. Summary of the significant changes ($P \leq .01$) in allele frequencies of cage populations I and IV (transferred to 15°C at generation 3).

	In nature		Brought into lab	Into cold 15°C		
Enzyme System	Sample original frequency	Cage	1	2	3	4
α Gpdh-3	+ = .9916	I	-	-*	-	-
	s = .0024					
	f = .0060	IV	-	-	-	-
Mdh-2	+ = .9760	I	-	-	-	-
	s = .0084					
	f = .0156	IV	-	-	-*	-
Me	+ = .9339	I	-	Inc. slow p= .01	-	-
	s = .0493					
	f = .0168	IV	dec. slow p=.001	-*	-	-
Idh	+ = .9724	I	-	-	-	-
	s = .0144					
	f = .0132	IV	-	-	-	-
Pgm	+ = .9411	I	-*	-	-	Inc. fast p=.001
	s = .0240					
	f = .0349	IV	dec. fast	-	Inc. fast p=.001	dec. fast p=.001

* = significance $.01 < p \leq .05$

s = slow allele f = fast allele + = most common allele

TABLE 4.4. Summary of the significant changes ($p \leq .01$) in allele frequencies of cage populations II and III maintained at 18°C.

	In nature		Brought into lab			
Enzyme System	Sample original frequency	Cage	1	2	3	4
α Gpdh-3	+ = .9916	II	-*	-	-	-
	s = .0024					
	f = .0060	III	-	-*	-*	-
Mdh-2	+ = .9760	II	inc. slow dec. fast p=.001	-	-	-
	s = .0084					
	f = .0156	III	-	-	-	inc. slow p=.003
Me	+ = .9339	II	-	inc. slow and fast p=.001	dec. fast p=.01	-*
	s = .0493					
	f = .0168	III	dec. slow and fast p=.001	-	inc. slow p=.01	dec.slow p=.001
Idh	+ = .9724	II	-	-	-	-
	s = .0144					
	f = .0132	III	-	-	-	-*
Pgm	+ = .9411	II	-	inc.slow p=.01	-	-
	s = .0240					
	f = .0349	III	-*	-	inc.fast p=.01	dec.slow and fast p=.001

* = significance $.01 < p \leq .05$

s = slow allele f = fast allele + = most common allele

ranging in the 70's. For a description of the various inversions found see Appendix II. Tables 3.12 through 3.16 give the frequencies found for the various arrangements. The heterozygosity is not distributed equally among the five chromosomes as can be seen in Table 4.5.

Chromosome A

All three known arrangements for Chromosome A, the shortest of the rod shaped chromosomes corresponding to the X chromosome, were found in Tuebingen. A 1 made up 27% of the gene arrangements.

Chromosome I

Only one small inversion, I 1 which includes the bulbioni ring, was found for Chromosome I. Initially, it made up close to 28% of the chromosomes sampled.

TABLE 4.5. Frequencies of heterozygotes per Chromosome.

	Chromosome					
	A (♀)	I	O	U	E	TOTAL FOR AUTOSOMES
Original	.45	.40	.48	.38	.20	1.46
Cage I_4	.34	.50	.45	.47	.16	1.58
Cage II_3	.45	.38	.58	.29	.30	1.55

Proportion of the genome involved in the structural heterozygosity is not considered.

Chromosome O

Seven different structural types were found for chromosome O ranging from the simple inversion O 6 to complex combinations of inversions, all of which contain O 3+4. Both O 3+4 and O 3+4+8 made up a significant proportion of the initial sample (14 and 10 percent, respectively). Gene arrangements O 3+4+8+2 and O 3+4+6 showed up in the final sample most probably

as recombinant types.

Chromosome U

Four inverted arrangements were found in chromosome U with U 1+2 at a frequency of over .?5. It was occasionally present in combination with U 6 or U 8 (4 and 3 percent, respectively).

Chromosome E

E 1+2 and E 8 both make up close to 5% of the structural types found. Gene arrangements E 1+2+9 and E 1+2+9+12 were found rarely.

Comparison of Chromosomal Arrangements with Other European Localities

Data collected from other European localities by Frutos (1975, in print), Knight (1961), Kunze-Muhl and Sperlich (1968 and 1962), and Prevosti (1964) indicate a north-south cline for the frequency of the standard arrangement for all five chromosomes. Table 4.6, 4.7, 4.8, 4.9 and 4.10 show the decrease of the standard arrangement as one moves southward, originally pointed out by Sperlich and Kunze-Muhl (1963) and Prevosti (1964). The Tuebingen data from this study corresponds well to the frequency of the standard arrangements as predicted by its latitudinal position.

Some interesting aspects of this north-south cline should be noted. Italy and Greece, separated from the rest of middle Europe by the alps have extremely low amounts of the standard arrangement. Southern France and Spain do differ by moderate amounts, through not as dramatically as perhaps one would expect considering the pyrenean border. On a limited regional scale, however, the effect of the barrier, as considered by Prevosti (1966), is definite. A 2 dominates in all three populations separated from middle Europe by mountain ranges (Spain, Italy and Greece). Although geographic boundaries have no doubt some effect, still it is improbable that

they are the sole cause of this dramatic clinal tendency.

The level of heterozygosity, as seen in Table 4.11 and 4.12 cooresponds to predictions based on findings of da Cunha and Dobzhansky (1954) and Carson (1955) about the decrease in structural heterozygosity found in marginal populations. Sperlich's data from 1964 for D. subobscura supports this view.

Scotland, which may be considered marginal and also somewhat isolated, seems to have a unique situation with U 1+2 dominating but still the average heterozygosity seen in Table 4.12 fits well into the general pattern.

4.2.2 Changes in Chromosomal Polymorphism in Laboratory Populations

There was no significant change in any of the five rod shaped chromosomes between the initial sample and cage II which was maintained at 18°C throughout the entire study period.

Likewise, there was no significant alteration in the karyotype frequencies for chromosomes A, E, and U between the initial sample and cage I which was changed to 15°C after generation 3. There was however a dramatic change in the proportions of I standard and I_1, in cage I. The inversion doubled its frequency thus being the most common chromosome I arrangement present in cage I at the end of the study.

The other significant change in arrangement proportions seen in cage I occurred in chromosome O where O 3+4+8 doubled its frequency at the expense of O 3+4.

Although no significant differences between the initial and later samples of the two cages were detected in chromosome A, U, and E, there was considerable divergence between the two cages

TABLE 4.6. Chromosome I arrangement frequencies for European populations of Drosophila subobscura.

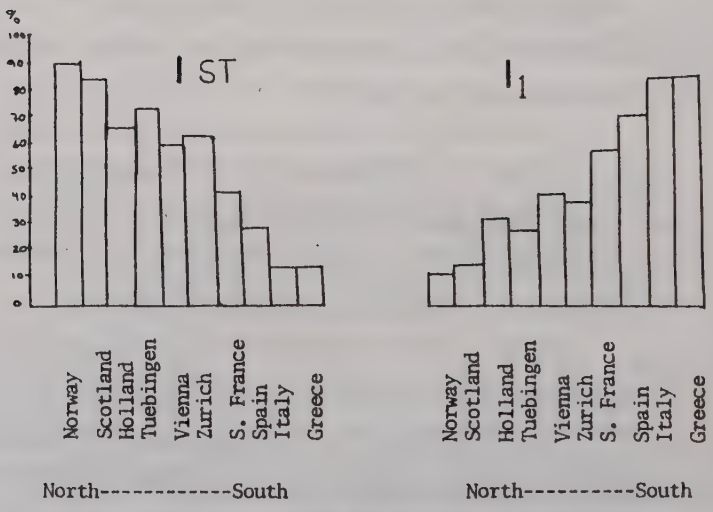


TABLE 4.7. Chromosome O arrangement frequencies for European populations of Drosophila subobscura.

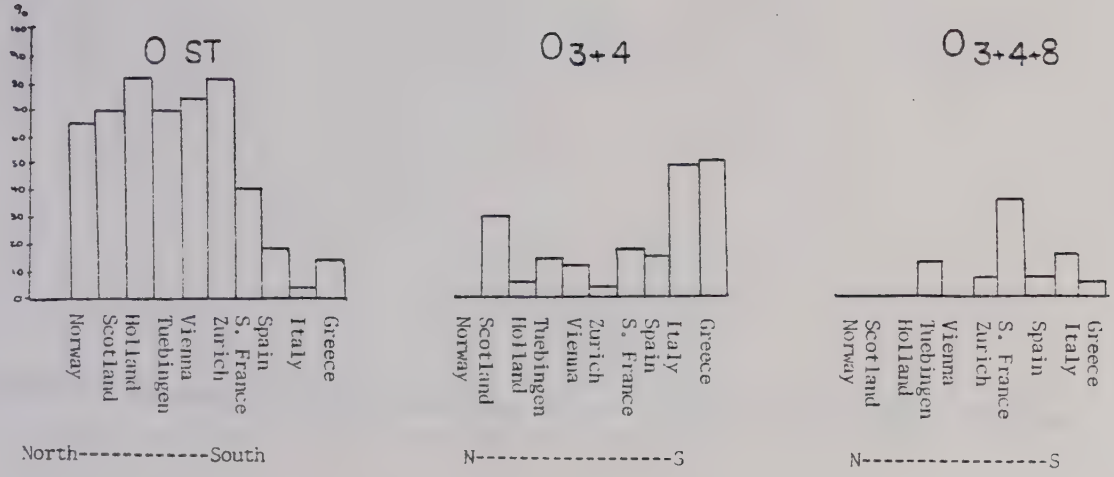
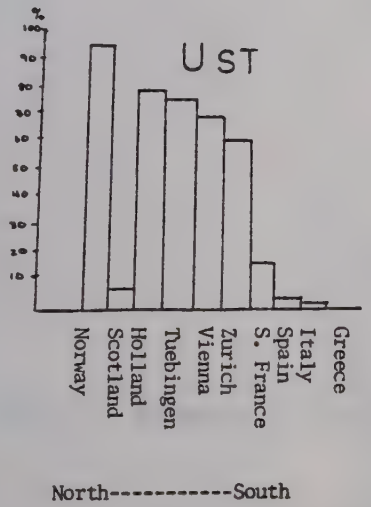
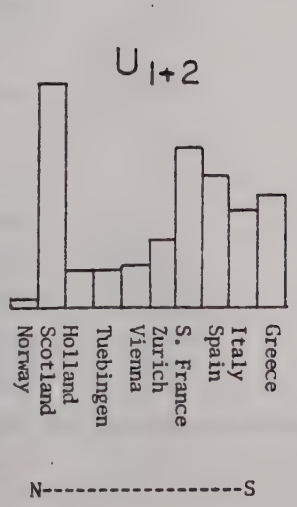


TABLE 4.8. Chromosome U arrangement frequencies for European populations of Drosophila subobscura.





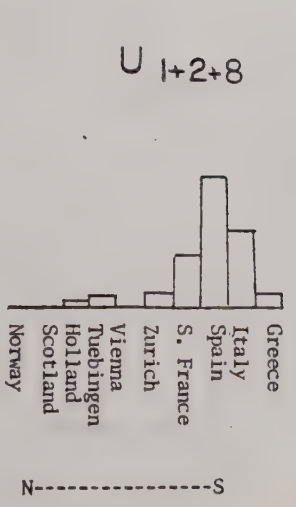


TABLE 4.9. Chromosome E arrangement frequencies for European populations of Drosophila subobscura.

TABLE 4.10. Chromosome A arrangement frequencies for European populations of Drosophila subobscura.

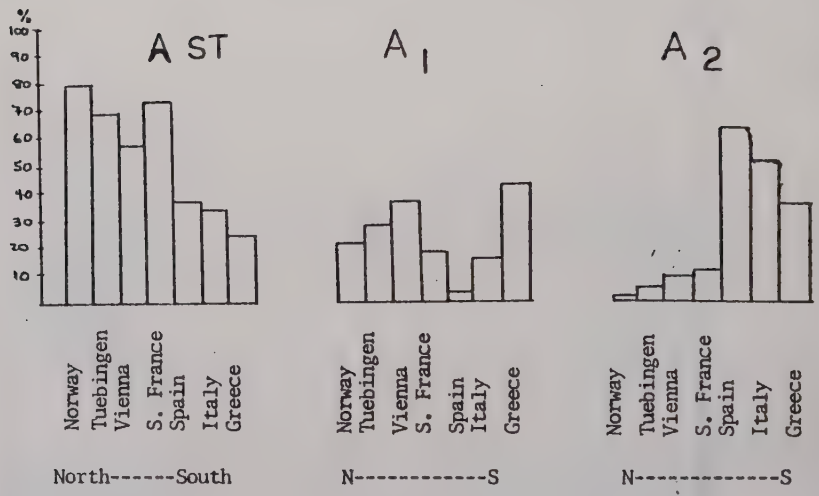


TABLE 4.11. Degree of chromosomal heterozygosity in Europe-individual chromosomes

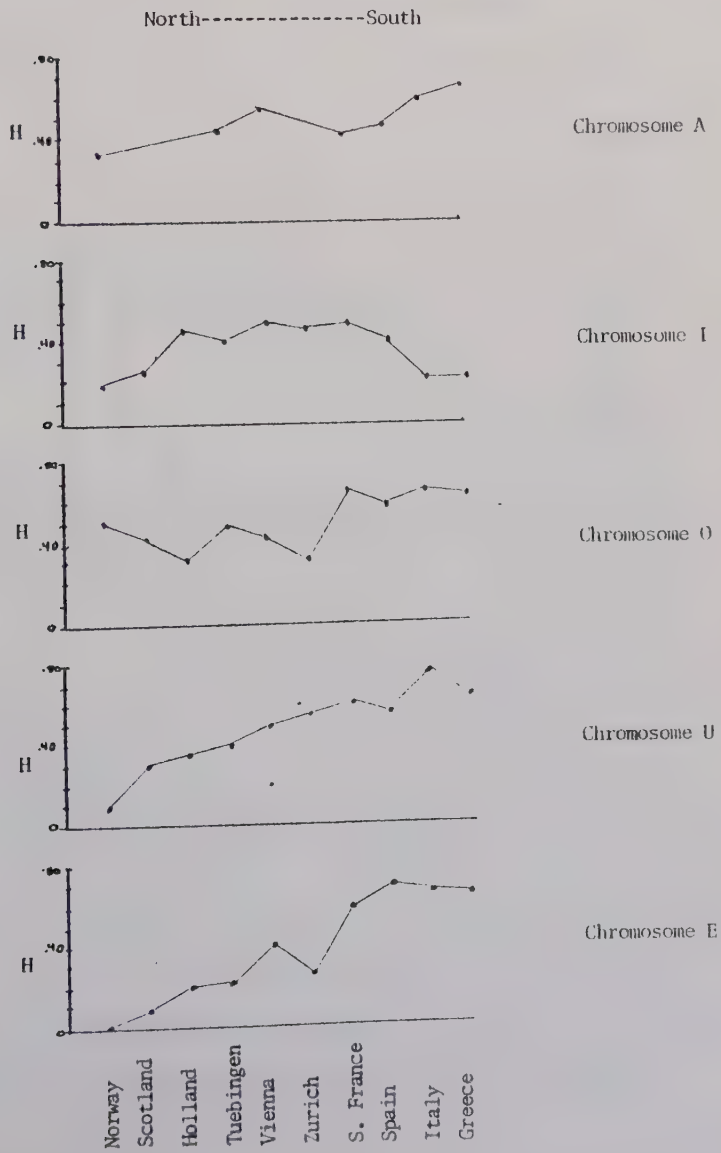
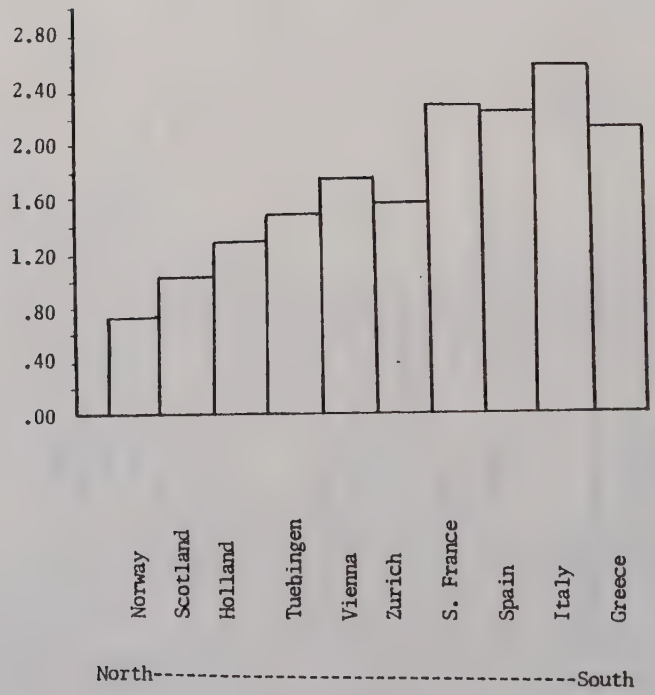


TABLE 4.12. Total degree of chromosomal heterozygosity in Europe



final samples as indicated by comparisons of cage I and cage II to each other. In cage I, U 1+2 and U 1+2+8 were more frequent. E 8 was, however, completely lost from this cage. This divergence is not surprising. One must remember these cages were founded with a great number of individuals from nature (1,664 wild chromosomes) carrying with them a large amount of genetic variation. Different recombinants will be formed by random process and selection will work with those combinations present in the population. The simple isolation of the two populations can therefore easily yield the small amount of divergence seen, although the cages do not differ in their A, E, and U chromosomes when compared to their original frequencies.

The changes seen in cage I in chromosome I and O, however are non-trivial. Chromosome I has only the one very small inversion, I_1. Others have concluded from their studies showing I to be stable, that perhaps too little genetic material is concerned to be greatly influenced by selection processes (Frutos, 1976). In this case, quality rather than quantity seems to be the case. Cage I has essentially maximised the number of heterozygotes for chromosome I arrangements with its final frequencies (the optimal frequencies being of course $p = 1/2$).

As in the Spanish study by Frutos, the flies kept under constant conditions in cage II, showed no change in the I chromosome arrangement frequencies.

An interesting phenomenon in the stability of chromosome A which also has simple inversions, but ones that include a considerable part of the chromosome. Despite this, and the fact that the hemizygous male condition disallows avoidance of selection pressure through

recessivity, no change was observed in the sex chromosome.

The O chromosome, on the other hand, has a complex inversion system. The increase of the O 3+4+8 at the expense of O 3+4 is quite obvious. It is interesting that in southern France the high frequency of O 3+4+8 is also accompanied by low levels of O 3+4 (Table 4.7).

Despite the changes seen in these two chromosomes, in general one would have to conclude that the chromosomal polymorphism was stable when maintained in the laboratory.

4.3 Association Analysis

4.3.1 Associations between Enzyme Alleles

Table 3.23 shows that the observed and expected numbers of individuals heterozygous for enzyme alleles in 1, 2, 3 or 4 of the enzyme systems studied in the larvae were significantly different. More than twice the number of individuals expected were heterozygous for two enzyme systems. When the allele associations among the different larval samples were analysed, only in the case of Mdh/Pgm combinations was a discrepancy from the expected values seen. Data for all combinations were pooled and presented in Table 3.24 through Table 3.29. The data for Mdh/Pgm combinations for each individual sample are then given in Tables 3.30 to 3.32. In both the initial sample and cage I, a significant difference between expected and observed numbers of individuals carrying both Mdh "fast" and Pgm "slow" is found. The pooled data for these two samples is seen in Table 3.33 (sign. = .001). This association obviously accounts for the overabundance of double heterozygotes found in the initial sample. In cage I, there were also more double heterozygotes than expected, but this was not enough to yield a significant chi square test.

One can see in Table 3.31, that this association in cage I accompanies a chi square value significant only at the .05 level.

It was therefore assumed that this association was brought in with the original flies from nature and was lost completely in cage II due to the initial loss of individuals with Mdh fast allele, while in cage I it has been maintained. Since these enzyme loci are located on different chromosomes, it is certainly not a case of linkage.

In order to test the hypothesis that this association was present in the original flies from nature, Mdh/Pgm combinations for the original 416 flies were analysed. As can be seen in Table 3.34, not one individual contained both Mdh "fast" and Pgm "slow". Likewise, the final adult sample for cage I shows no association between these two alleles. With both adult samples the frequency of both Mdh "fast" and Pgm "slow" is lower than frequencies obtained from larval analysis. If one then compares the data obtained for larval and adult analysis from the same sample, statistically significant differences between Mdh allele and Pgm allele frequencies are found. Table 4.13 shows that this is not the case with the other two enzyme systems and also not with Mdh and Pgm in cage II where this association does not exist. In both cages where the association is present among the larvae, initially and later in cage I, there is a significantly higher proportion of Mdh "fast" and Pgm "slow" than in the adults from the same sample. Curiously, this association between Mdh "fast" and Pgm "slow" alleles was never found in any adult sample.

One possible explanation could be concerned with larval behavior. If Mdh-"f"/Pgm-"s" larva come out of the medium more

TABLE 4.13. Comparison of enzyme allele frequencies for larval and adult analysis of the same sample.

Comparison of samples	Enzyme System	X^2	d.f.	Significance
Reduced Initial (larval) with I_1 and II_1 (adult)	Mdh-2	21.1388	1	.001
	Pgm	12.8796	1	.01
	Me	2.9930	1	not sign.
	Idh	no data		
Cage I_4 (larval)	Mdh-2	25.5056	1	.001
with I_4 (adult)	Pgm	29.0168	2	.001
	Me	.5016	1	not sign.
	Idh	.0826	1	not sign.
Cage II_3 (larval)	Mdh-2	2.7528	1	not sign.
with II_3 (adult)	Pgm	1.6667	2	not sign.
	Me	.4825	1	not sign.
	Idh	.0255	1	not sign.

frequently, they may be chosen for analysis more frequently than other genotypes. This could result in the overabundance of Mdh-"f"/Pgm-"s" individuals among larvae. In cage II, where this overabundance of Mdh-"f"/Pgm-"s" combinants was absent, there were much lower levels of both these rare alleles at the end of the study. Thus it is not surprising that in the final sample for this cage, the differential larval behavior did not signifianctly alter the proportion of combinations from those expected. There were simply too few Mdh-"f"/Pgm-"s" individuals to alter the results.

Sperlich, Pinsker and Abindin (1975) have previously reported an association between the Mdh-2 loci and U chromosomes inversion system in a laboratory population of D. subobscura originally from Tunis. In these flies, the Mdh-2 'plus' allele (.96) was associated with U 1+2, with the fast allele (1.05) accompaning individuals carrying U 1+2+8. Sperlich concluded from his work that the Mdh-2 polymorphism was neutral and hitchhiking along with the inversion polymorphism known to be under the influence of natural selection. He therefore assumes that this association will not always be present but rather is incorporated into the gene pool depending on its individual selective effect. This association was not found in the flies from Tuebingen, although both Mdh alleles were present. It is of course well established that particular gene arrangements from different geographic locations often contain a different set of coadapted genes (Dobzhansky, 1948). Pinsker, Lankinen, and Sperlich (1978), however, recently studied D. subobscura in Tuebingen and once again found a non-random association between the rare allele 1.05 of Mdh ('fast') and U 1+2+8, and to a lesser extent with U 1+2. Although the frequency of the Mdh allele 1.05 in both

this study and theirs is the same, they report a much higher frequency of the U 1+2 arrangement (24% as opposed to 15%) than was found in the present study. The same can be said for U 1+2+8 (5% as opposed to 2.8%). Thus it is possible that sampling error accounts for the absence of this association in the present study.

In this same study, Sperlich did observe a difference in allelic frequencies between the larvae and adults not totally explainable by the heterosis present. There was also a deviation from the expected equilibrium values based on calculations with relative selective values. This he attributed to random drift.

With the observations from the present study where the frequency of the fast allele is much greater in the larvae, only to disappear in the adults, and yet reappear in the next larval sample again at high levels thus eliminating an explanation based on selective forces, it seems reasonable to assume, one is actually looking at two closely linked genes such that alleles detected in larvae and adults for Mdh-2 are actually the products of different loci. Due to the close linkage, detection from the relatively few single pair matings used to determine allelism would not be possible since the various cross over recombinations would not be expected to appear with such small numbers.

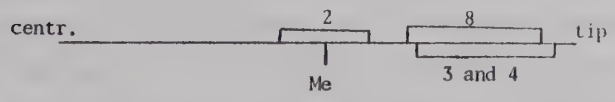
However, a series of experiments by Pinsker definitely established the Mdh-2 locus to be on chromosome U while Pgm is on Chromosome I. The Mdh-1 Locus known also to stain in larvae always runs cathodally and is monomorphic. Thus a more plausible explanation is that of differential larval behavior already mentioned,but the problems remains open.

4.3.2 Associations between enzyme alleles and chromosomal arrangements.

Combinations of enzyme alleles and various structural arrangements

were analysed for each separate larvae sample with significant deviations from the expected numbers arising only with the intra-chromosomal association between Me/chromosome O. Data for all associations proven to be in accordance with expected results in each separate larval sample were pooled and still results in non-significant chi square values. Of course, non-random associations of dissociations were not expected for interchromosomal combinations.

The association found to exist between Me slow and O 3+4+8 is present both in cage I and cage II, although absent from the initial sample. Thus, here the association was not brought in with the flies from nature but rather developed independently in both of the cages. Although this association is significant only to the .05 level in each age, combined data is significant to .01 level. The Me loci is located on the O chromosome but is independent of the O 3+4+8 inversions (see Figure 4.1 (Zouros et al, 1974)).



The Me locus ishowever located within the O 2 inversion (see Appendix II). It is interesting to note that the one case of O 3+4+8+2 also was associated with Me slow althought O 3+4+2 was not. Although O 3+4+8 doubled its frequency in cage I, the frequency of Me slow remained constant in both cages when larval data was compared to the original sample. (See table 3.19 and chi square values in 3.20.) Thus although the association exists in each cage, it is not particularly strong.

This overabundance of individuals carrying both the Me-slow allele and the O 3+4+8 gene arrangement is most probably due to an

advantage conferred upon these individuals. The selection takes place between the egg and 3rd instar larval stage. This seems a more likely explanation than non-random sampling due to larval behavior (as is the case with the non-random Mdh-/Pgm combinations), since the initial larval sample showed random combinations of Me alleles and O chromosomal arrangements despite the high frequencies of both Me-slow alleles (>10%) and O 3+4+8 karyotypes (>10%).

4.3.3 Association between chromosomal arrangements

The number of individuals heterozygous for inversions in various numbers of autosomes (see table 3.37) is as would be expected from their relative frequencies. It is therefore, not surprising that combinations of structural arrangements from different chromosomes are not significantly different from expected values. This held true for each separate larval sample. The pooled data for all larval analyses (tables 3.38 to 3.43) also yield insignificant chi square values, thus indicating the various gene arrangements on the different chromosomes are combined randomly.

Sperlich and Feuerbach have shown (1969 and 1974) that among the independent inversions of the same chromosome often times a strong deviation from randomness is observed. In fact, there is complete linkage disequilibrium for almost all independent inversions in natural populations studied. In this particular study, A 1 was never found with A 2. O 3, O 4, O 8 and O 2 were never seen alone, but rather complexes of O 3+4, O 3+4+8, and these two complexes in combination with O 2 or O 6. U 2 was always found in the complex U 1+2. U 6 and U 8 were always associated with U 1+2. In chromosome E, E 1 and E 2 were always together with each other, and often with

E 9 or E 9+12. Although this study's data alone could not be used to investigate this matter, it certainly supports Sperlich's findings. It is interesting that O 3+4+8+2 did appear once as a result of recombination. This proves that recombination does take place between O 3+4 and O 2. O 3+4+8+2 has, however, never been found in nature.

CHAPTER FIVE

CONCLUSIONS

5.1 Enzyme Polymorphism in nature

In all five enzyme systems investigated, multiple alleles were found. Various systems exhibited different levels of polymorphism with malic enzyme and phosphoglucomutase having the most variability. These moderate amounts of enzyme polymorphism are consistently seen throughout Europe.

5.2 Stability of Enzyme Polymorphism

In general, one can say that the enzyme polymorphism in Drosophila subobscura is very stable when brought into the laboratory, and subjected to different environmental regimes. Most fluctuations could be attributed to random processes with the usual response of the populations resulting in fluctuations around initial frequencies or stable maintenance of the new frequency.

5.3 Chromosomal Polymorphism in Nature

A highly developed inversion polymorphism is present in the Tuebingen population. The most common arrangement found in each of the five acocentric chromosomes is the standard order. Chromosomes A, I and O show the highest levels of heterozygosity yet exhibit different inversion pattern phenomenon. Both A and I have only single inversions, those in A comprising a considerable part of the genome, while the one inversion present on I includes only the immediate area around the balbiani ring. Chromosome O, on the other hand has a complex system of tandem and overlapping inversions involving large parts of the chromosome. Chromosomes U and E systems are

also made up of complex inversions but the level of heterozygosity is lower in these chromosomes. Nevertheless, the high levels of inversions in the five chromosomes insures homozygosity for the entire genome to be extremely rare.

The pattern of inversion polymorphism is not similar throughout Europe as was seen for enzyme polymorphism. Instead, we have a situation where the level of heterozygosity decreases in marginal populations, as does the frequency of the standard arrangement in all five chromosomes following a distinct north-south cline. More information is needed before one could explain the origin and existence of these geographical clines. It seems improbable that historical or geographical causes are the only factors involved. On the other hand temperature as the selective factor seems unlikely. Further investigations of other environmental parameters are needed.

Theoretical arguments assume that marginal populations with their more limited range of niches have less need for a highly developed homeostasis ability but rather would fend better with more specialized individuals. This agrees with the high frequency of the standard arrangement seen in all of the autosomes in the northern European populations. Central populations like Tuebingen would benefit more greatly from a system conferring great homeostasis ability on the individuals within a population thus allowing them to exploit the wide range of niches available to them. Sperlich (Sperlich et al., 1976) has pointed out that this concept that the heterotic action of inversions is due to homeostatic capabilities conferred on their carriers helps to explain the reduced inversion polymorphism seen in marginal populations in spite of lack of such a reduction in genetic polymorphism. The decrease in inversions in the marginal

populations is of course associated with an increase in phenotypic plasticity.

5.4 Stability of Chromosomal Polymorphism

In general, the chromosomal polymorphism remained stable despite environmental manipulations. As an explanation for the stable type of chromosomal polymorphism, Dobzhansky (1962) suggests that selection is for the genotype that is buffered against any changes that would occur in the environment. Thus through their homeostatic ability, individuals in a population could thrive in the various environmental conditions available without having to drastically change the population's structural composition. This buffering ability, however, is most certainly not infinite. It is, therefore, possible that large differences seen over great geographical distances cannot be coped with in the usual way and therefore one does see significant changes when comparing different localities.

In species with a flexible system, the different karyotypes present in the population would each be specially adapted to a set of environmental conditions and thus in response to a change in these conditions, the population would undergo a shift in the relative proportions of its 'specialists'. In the population of Drosophila subobscura from Tuebingen, one rarely finds a complete homokaryotypic individual. Only in chromosome I and O was a significant shift from the original values observed.

Krimbas (1964) has suggested that the main selective agent in nature controlling the gene arrangement frequencies are temperature and humidity but further studies (Krimbas, 1967) did not support

this hypothesis. But as in all investigations of selective advantage, a negative results does not prove the absence of selection. The dramatic increase in I 1 cannot simply be attributed to the cooler temperature experienced in that cage since it is actually in the warmer European localities that I 1 frequencies are highest. Nevertheless, the change can be considered a meaningful response of the population to the change in its environment; a response which in fact maximizes the number of heterozygotes for this chromosome. There is evidence supporting the greater fitness of heterozygotes for chromosome I (Krimbas, 1967, Pentzos-Daponte, 1964, Kunze-Mühl, and Mueller and Sperlich, 1958).

The increase in O 3+4+8 implies an advantage conferred to individuals with repressed recombination within the region of this inversion. The association of this inversion with an enzyme allele whose locus lies outside of the inverted region, but is associated only with the O 3+4+8 combination and not the O 3+4 order implies perhaps an influence dependent on position effect. This association developed independently in both cages, thus eliminating the explanation of random processes taking place in the founding population. Sperlich and co-workers (1977) have suggested that "the effect of inversions, or better of the genetic information locked together inside of them, may not depend so much on the structural genes but on a higher level of organization which may be interaction or regulation together with position effects (Sperlich, 1966) to name a few." From the findings in this study, I would suggest that the advantage conferred on individuals carrying the O 3+4+8 inversion is due to position effects since this inversion is associated with the Me slow allele from a locus outside the inverted region (see

Zouros et al, 1974). The Me slow is not associated with the O 3+4 order. In fact, although the Me locus is located within the small O 2 inversion, the few cases of O 3+4+2 showed no association with Me slow, while the one individual with O 3+4+8+2 also carried this allele, adding strength to this hypothesis.

When one further considers that according to recent estimates, less than 10 percent of the DNA in eukaryotic genomes is involved with genes that are transcribed and translated to produce functional enzymes and proteins, with the rest assumably concerned with regulation (Selander, in Ayala 1976); and that man and chimp share over 99% of their structural genes and yet are quite differentiated with respect to their common ancestor, it is natural to suggest that possibly the important changes in the genome for evolution are those concerned with gene regulation (Wilson et al, 1974, 1975). Further studies investigating the position effect of inversions would be extremely instructive.

5.5 Enzyme allele interactions

Only in Mdh and Pgm was an association found between two alleles, and then only in the larvae. This was most probably due to selection or larval behavior. Similar findings by Zouros et al (1974), and Saura et al (1973) indicate disequilibria among enzyme loci alone to be rare in Drosophila subobscura. Because of the incomplete discrimination of alleles by electrophoreses such an association is not likely to be observable, however.

5.6 Chromosomal interactions

It is thought that inversions are selected for since they would thereby maintain the integrity of coadapted gene blocks. If one

assumes that selection would favor linkage of interacting genes, then one could consider chromosomes themselves, as linkage groups, functional and selectional units. The finding of no interaction between inversions on different chromosomes yet complete linkage disequilibrium among some inversions on the same chromosome would seem to support this assumption. There is no reason to assume that the genes entrapped within a specific inversion in one locality are necessarily the same in another area. In fact, the opposite seems to be supported by investigation of total linkage disequilibrium among intrachromosomal arrangements. Sperlich and Feuerbach (1974) have suggested the absence of A 1+2 despite a high frequency of both independent inversions and proof of crossover between the two is due to lethal position effect. The evolution of the inversion complexes took place of course concurrently with the evolution of genic polymorphic systems. The difference in the genic composition of the various arrangements presumably reflects the historical development of the gene blocks under the conditions prevalent in that location during this time.

5.7 Associations between enzyme alleles and chromosomal arrangements

Only with O 3+4+8 and Me-slow, was an association found. This has already been interpreted as a position effect. All other alleles were randomly combined with chromosomal arrangements. Since this one association was found in both end samples but not in the original flies, it is not simply due to random founding effect. Neither can it be attributed to any difference in environment between the two cages (15° versus 18°) since both cages show a significant deviation from expected to the .05 level. This association is however weak

since a doubling of the O 3+4+8 in cage I was not accompanied by an equal response in Me slow.

5.8 Summary

A study of four cage populations founded by 416 inseminated Drosophila subobscura females from a natural population in Tuebingen, West Germany showed the natural population to have moderate amounts of genic polymorphism in the enzyme loci Mdh, Idh, Pgm and Me in line with frequency levels seen consistently throughout Europe. The population maintained these frequencies in the laboratory despite having been subjected to different temperature regimes, with slight amounts of fluctuation being observed among the more polymorphic of the five loci studied.

A highly developed chromosomal polymorphism was detected which fit in well to a general north-south cline for the frequency of the standard arrangement for each chromosome seen throughout Europe. In all cases, this standard arrangement was the most common karyotype present. No change was seen in a cage maintained under constant conditions, but a cage changed to cooler temperatures after generation 3 underwent a drastic increase in gene arrangements I 1 and a doubling of the frequency of chromosome type O 3+4+8 at the expense of O 3+4. The change in the I chromosome arrangement frequencies maximised the number of I st./I 1 heterozygotes shown to have a selective advantage in other studies. The increase of O 3+4+8 at the expense of O 3+4 was attributed to an advantage to restricting recombination in this segment of the genome. An association between O 3+4+8 with a rare Me slow allele (not located within the inverted region) was interpreted as being due to some

sort of position effect interaction. This association was most probably not established by random sampling among the founders but rather developed independently in two laboratory populations.

The only other non-random combination found was one between Mdh-fast allele and Pgm-slow allele leading to higher levels of both these alleles in the cage population containing this association. It was however, only found among the larvae of the initial and end samples of one cage, never among the adults. Since adult data from the same sample showed significantly lower levels of both alleles combined, it is suggested that larval behavior might have led to a selective sampling procedure.

Complete disequilibrium among independent inversions found previously (Sperlich and Feuerbach, 1969) is supported by this study's data too. The problems of neutral versus selective hypothesis are discussed with respect to our results.

APPENDIX I

Advice on statistics for this study by Professor J. David Mason, University of Utah, Mathematics Department, is gratefully acknowledged.

STATISTICAL ANALYSIS OF DATA

1. Chi square tests were computed according to the standard formula

$$X^2 = \sum_{i=1}^{K} \frac{(O_i - E_i)^2}{E_i}$$

where

O_i = observed frequencies

E_i = expected frequencies

2. 2 x 2 contingency tables were computed using the standard formula

$$X^2 = \frac{(|ad-bc| - \frac{1}{2}n)^2 n}{(a+b)(a+c)(b+d)(c+d)}$$

where

$n = a+b+c+d$

a and b = absolute frequencies in one sample

c and d = absolute frequencies in second sample

3. Larger contingency tables were computed using the standard m x n table formula

$$X^2 = \left(\sum_{i=1}^{m} \sum_{j=1}^{n} \frac{a_{ij}^2}{\left(\sum_{k=1}^{n} a_{ik} \right) \cdot \left(\sum_{r=1}^{m} a_{rj} \right)} - 1 \right) \left(\sum_{i=1}^{m} \sum_{j=1}^{n} a_{ij} \right)$$

where

m = number of rows

n = number of columns

each row corresponds to absolute frequencies of one sample

each column corresponds to a specific allele or gene arrangement

a_{ij} = entry in ith row and jth column

4. Due to the small sizes of particular classes, it was occasionally necessary to use a test for normal distribution of proportions in two independent samples. Normal contingency tables were not adequate since either more than 20% of the expected values were less than five, or any expected value was less than one.

The following standard formula was used

$$Z = \frac{(p_1 - p_2)}{\sqrt{\bar{p}(1-\bar{p})\left(\frac{1}{n_1} + \frac{1}{n_2}\right)}}$$

where

p_1 = probability (relative frequency) in sample one

p_2 = probability in sample two

n_1 = number in sample one

n_2 = number in sample two

$$\bar{p} = \frac{n_1 p_1 + n_2 p_2}{n_1 + n_2}$$

p level was 2 x (1-Z)

APPENDIX II

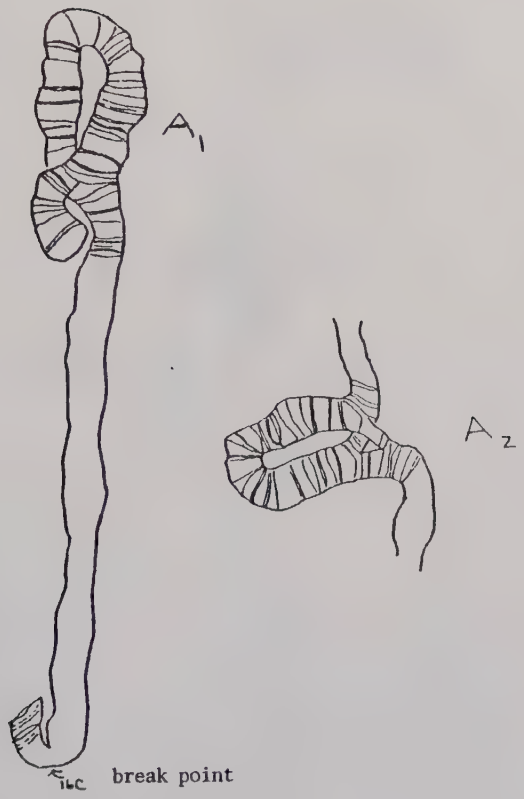
CHROMOSOME INVERSIONS

CHROMOSOME	COMPRISES AREA BETWEEN	INVERSION	EXTENDS FROM	STRUCTURE TYPES
A	1A to 16 D	A_1	2A/2B to 7C/7D	$A_{st.}$
				A_1
				A_2
		A_2	8C/8D to 12A/12B	
I	17A to 35E	I_1	22B/22C to 25A/25A	$I_{st.}$
				I_1
O	75A to 99C	O_2	85C/85D to 89B/89C	$O_{st.}$
				O_{3+4}
		O_3	91B/91C to 94E/95A	O_{3+4+8}
				$O_{3+4+8+2}$
		O_4	94D/94E to 98C/98D	O_{3+4+2}
				O_{3+4+6}
		O_6	85B/85C to 90C/90D	O_6
		O_8	90D/91A to 94A/94B	

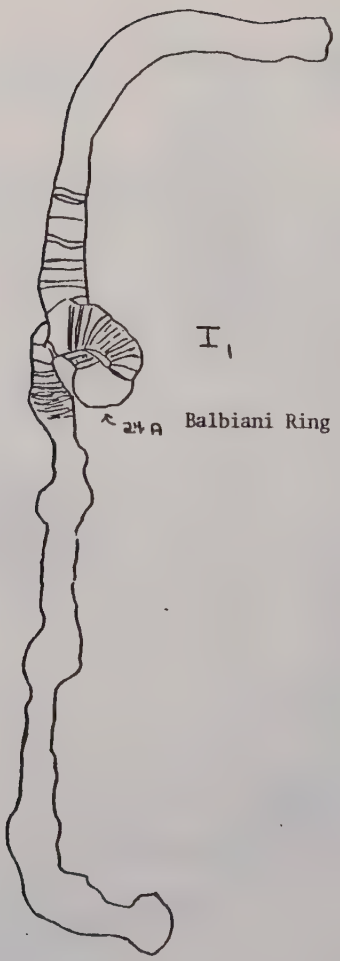
CHROMOSOME	COMPRISES AREA BETWEEN	INVERSION	EXTENDS FROM	STRUCTURE TYPES
U	36A to 53D	U_1	39D/40A to 45E/46A	$U_{st.}$ U_1
		U_2	40A/46A to 51C/51D	U_{1+2} U_{1+2+6}
		U_6	45A/45B to 49D/49D	U_{1+2+8}
		U_8	40A/51C to 44D/45A	
E	54A to 74D	E_1	58D/59A to 62D/63A	$E_{st.}$ E_{1+2}
		E_2	58D/62A to 64B/64C	E_{1+2+9} $E_{1+2+9+12}$
		E_8	61C/61D to 68D/68E	E_8
		E_9	58D/64B to 68B/68C	
		E_{12}	61C/61D to 67A/67B	

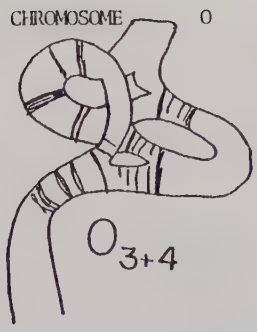
taken after Kunze-Muehl and Mueller, 1958
Stumm-Zollinger, 1953
Goetz, W., 1965

CHROMOSOME A

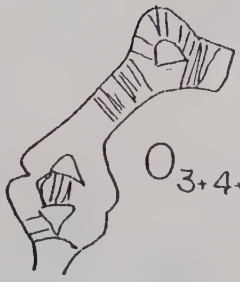


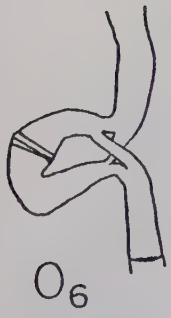
CHROMOSOME I

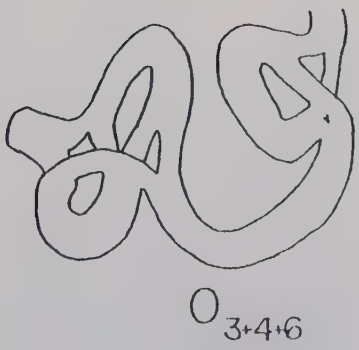



CHROMOSOME O
O3+4

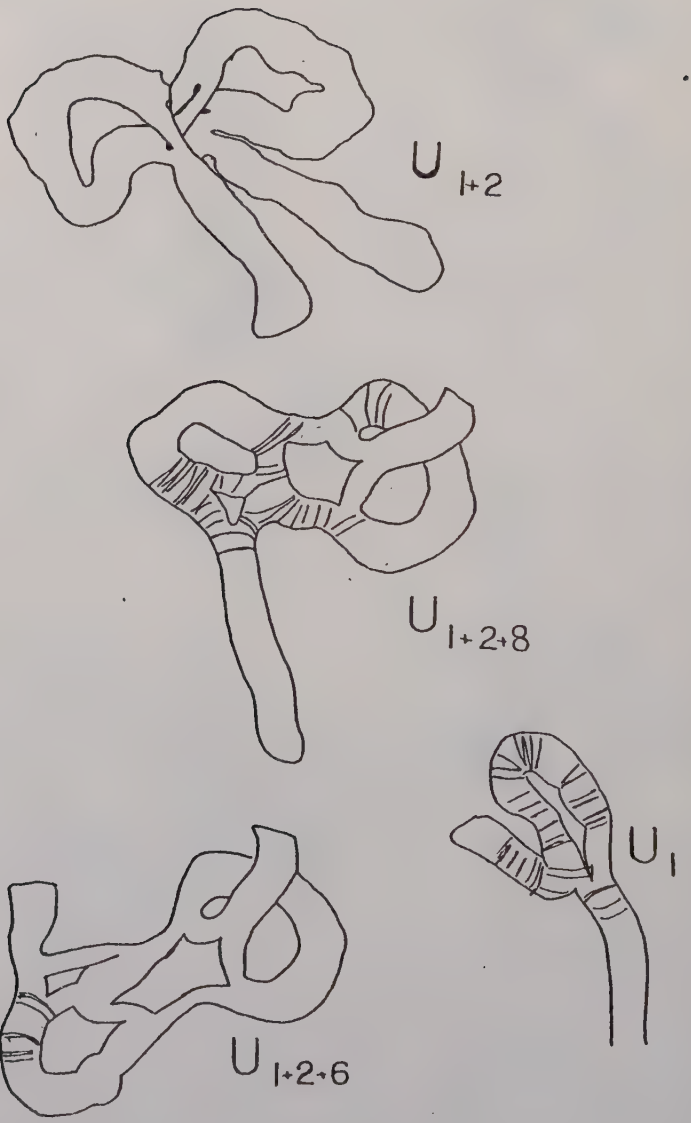
O3+4+8 (paired)


O3+4+8 (unpaired)

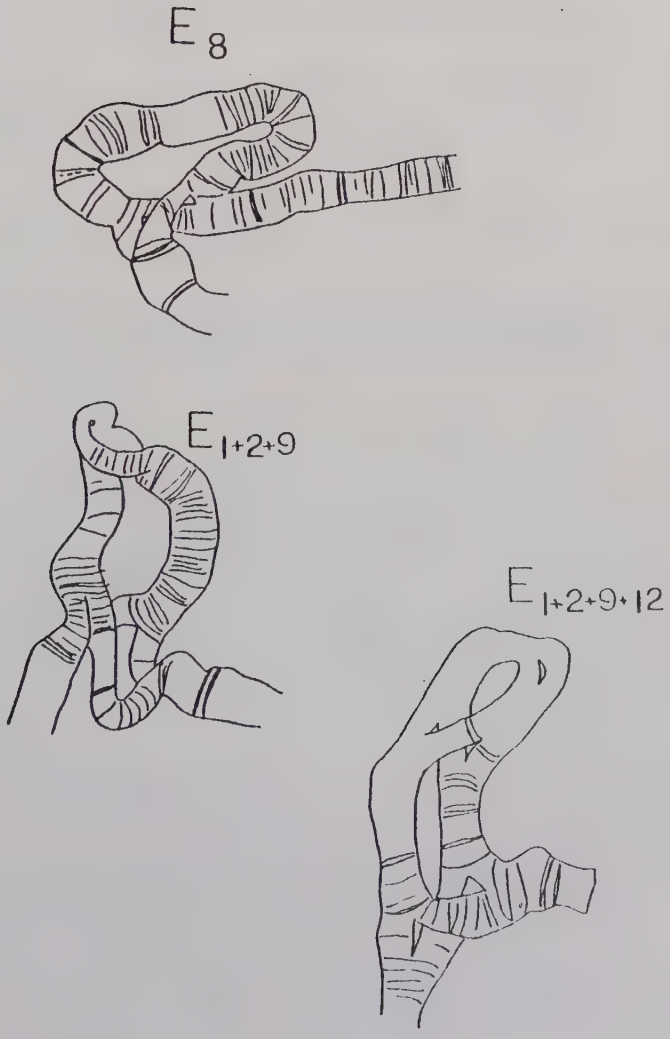

O6


O3+4+6

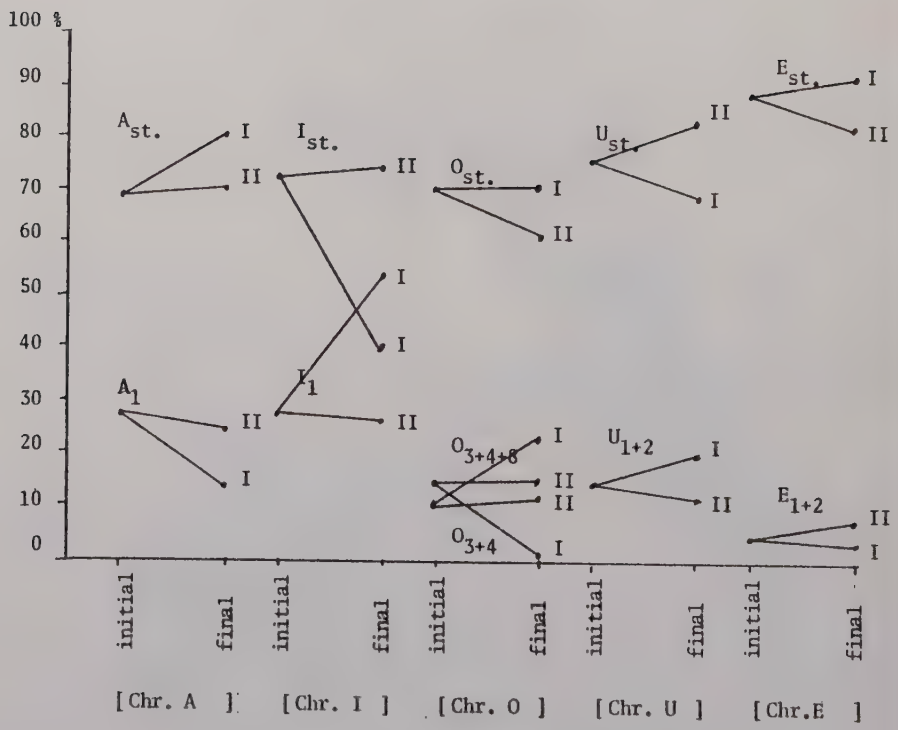
CHROMOSOME U



CHROMOSOME E



Summary of changes in chromosome arrangement frequencies between initial and final samples from experimental population cages I and II. 138



BIBLIOGRAPHY

1. Anderson, W., C. Oshima, T. Watnabe, Th. Dobzhansky and O. Pavlovsky. 1968. Genetics of natural populations XXXIX: A test of the possible influence of two insecticides in the chromosomal polymorphism in Drosophila pseudobscura; Genetics, 58; 423-434.

2. Ayala, F.J., A. Mouraõ, S. Perêz-Salas, R. Richmond and Th. Dobzhansky. 1970. Enzyme variability in the Drosophila willistoni Group, I. Genetic differentiation among sibling species; Proc. Nat. Acad. Sci., 67; 225-232.

3. Ayala, F.J., J.H. Powell and Th. Dobzhansky. 1971. Polymorphisms in continental and island populations of Drosophila willistoni; Proc. Nat. Acad. Sci. USA, 68; 2480-2483.

4. Ayala, F.J., J.R. Powell, M.L. Tracey, C.A. Mouraõ and S. Perêz-Salas. 1972. Enzyme variability in the Drosophila willstoni Group, IV. Genic variation in natural populations of Drosophila willistoni; Genetics, 70; 113-139.

5. Ayala, F. 1976. MOLECULAR EVOLUTION. Sinauer Assoc. Inc., Sunderland, Mass.

6. Bernstein, N. and E. Goldschmidt. 1961. Chromosome breakage in structural heterozygotes; Am. Naturalist, 95; 53-59.

7. Brncic, D. 1961. Non-random association of inversions in Drosophila pavani; Gen. 46; 401-406.

8. Burla, H. and W. Götz. 1965. Veränderlichkeit des chromosomalen Polymorphismus der Drosophila subobscura; Genetica, 36; 83-104.

9. Carson, H.L. 1955. The genetic characteristics of marginal populations of Drosophila; Cold Spr. Harb. Symp. Quant. Biol., 20; 276-287.

10. Carson, H.L. 1955. Variation in genetic recombinations in natural populations; Comp. Physiol., 45 surpl. 2; 221-236.

11. Cunha, D. da and Th. Dobzhansky. 1954. A further study of chromosomal polymorphism in Drosophila willistoni in its relation to the environment; Evolution Lancaster, Pa 7; 119-134.

12. Cunha, B. 1955. Polymorphism in Diptera; Advanc. Genet. 7; 93-138.

13. Dobzhansky, Th. 1948. Genetics of natural populations XVIII: Experiments on chromosomes of Drosophila pseudoobscura from different geographic regions; Genetics, 33; 588-602.

14. Dobzhansky, Th. and O. Pavlovsky. 1953. Indeterminate outcome of certain experiments on Drosophila populations; Evol. 7; 198-200.

15. Dobzhansky, Th. and N.P. Spassky. 1962. Genetic drift and natural selection in experimental populations of Drosophila pseudoobscura; Proc. W.A.S., Vol. 48; 148-156.

16. Dobzhansky, Th. 1970. GENETICS OF THE EVOLUTIONARY PROCESS. Columbia University Press, N.Y.; 505 pp.

17. Götz, W. 1965. Beitrag zur Kenntnis der Inversionen, Duplikationen und Strukturtypen von Drosophila subobscura; Z. Vererbungsl, 96; 285-296.

18. Hubby, J.L. and L.H. Throckmorton. 1968. Protein differences in Drosophila, IV. A study of sibling species; Amer. Natur., 102; 193-205.

19. Hunter, R.L. and C.L. Markert. 1957. Histochemical demonstration of enzymes separated by zone electrophoresis in starch gels; Science, 125; 1294-1295.

20. Jungen, H.E. 1968. Inversionspolymorphismus in tunesischen Population von Drosophila subobscura; Archiv. J. Klaus-stiftg., 43; 3-55.

21. Knight, J.R. 1961. Structural polymorphism in Drosophila subobscura Coll. from various localities in Scotland; Gen. Res., 2; 1-9.

22. Krimbas, C. 1964. The genetics of Drosophila subobscura populations. I. Inversion polymorphism in populations of southern Greece; Evolution, 18; 541-552.

23. Krimbas, C. 1967. The genetics of Drosophila subobscura populations. III. Inversion Polymorphism and climatic factors; Molec. Gen. Genetics, 99; 133-150.

24. Kunze-Mühl, E. and D. Sperlich. 1955. Inversionen und chromosomale Strukturtypen bei Drosophila subobscura; Z. Ind. Abst. Vererb., 87; 65-84.

25. Kunze-Mühl, E., E. Miller and D. Sperlich. 1958. Qualitative, quantiative und jahreszeitliche Untersuchungen über den chromosomalen Polymorphismus natürlicher Populationen von Drosophila subobscura; Coll. in der Umgebung von Wein Zeits. Vererbungsl., 89; 636-646.

26. Kunze-Mühl, E. and D. Sperlich. 1962a. Vergleichende Untersuchungen über den chromosomalen Polymorphismus natürlicher Populationen von Drosophila subobscura; Z. Vererbungsl., 93; 237-248.

27. Kunze-Mühl, E. and D. Sperlich. 1962b. Vergleichende Untersuchungen über den chromosomalen Strukturpolymorphismus in Insel - und Festlands Populationen von Drosophila subobscura; Zert. fur. Vererbungsl., 93; 237-248.

28. Lakovaara, S. and A. Saura. 1971. Genetic variation in natural populations of Drososphila obscura; Gent, 69; 377.

29. Lakovaara, S., A. Saura and C.T. Falk. 1972. Genetic distance and evolutionary relationships in the Drosophila obscura Group; Evolution, 26; 177-184.

30. Lakovaara, S., A. Saura, P. Lankinen and J. Lokki. 1972. Evolution of enzymes and genetic distance in Drosophila obscura and affinis subgroups; XVII^{e} Congrès International de Zoologie Monte Carlo, September 1972; Theme No. 5, Etudes moleculaires des différences entra les espècies.

31. Lankinen, P. and W. Pinsker. 1977. Allozyme constitution of two standard strains of Drosophila subobscura; Sep. Experientia, 33; 1301.

32. Levene, H., O. Pavlovsky and Th. Dobzhansky. 1958. Dependence of the adaptive values of certain genotypes in Drosophila pseudoobscura or the composition of the gene pool; Evolution, 12; 18-23.

33. Lewontin. R.C. 1974. THE GENETIC BASIS OF EVOLUTIONARY CHANGE. Columbia University Press, N.Y.

34. Nair, P.S., D. Brncic and K. Kojima. 1971. II Isoenzyme variations and evolutionary relationships in the mesophragmatica species group of Drosophila; University Texas Publ., 7103; 17-28.

35. Pavlovsky, O. and Th. Dobzhansky. 1966. Genetics of natural populations XXXVIII. The coadapted system of chromosomal variants in a population of Drosophila pseudoobscura; Genetics, 53; 843-854.

36. Pentozos-Daponte, A. 1964. Qualitative und quantitative Untersuchungen über den chromosomalen Polymorphismus natürlicher Populationen von Drosophila subobscura in der Umgebung von Thessaloniki-Griechenland; Z. Vererbungsl., 95; 129-144.

37. Pentozos-Daponte, A. and D. Sperlich. 1965. Vitalitäts Prüfungen an Intra und interlokalen Heterozygoten von Drosophila subobscura; Z. Vererbungsl., 97; 46-54.

38. Pinsker, W., P. Lankinen and P. Sperlich. 1978. Allozyme and inversion polymorphism in a central european population of Drosophila subobscura; Genetica Vol. 48, 3; 000-000.

39. Prakash, S. 1973. Patterns of gene variation in central and marginal populations of Drosophila robusta; Genetics, 75; 347-369.

40. Prevosti, A. 1964. Chromosomal polymorphism in Drosophila subobscura populations from Barcelona (Spain); Genet. Res., 5; 27-38.

41. Prevosti, A. 1966. Chromosomal polymorphism in western mediterranean populations of Drosophila subobscura; Genet. Res. Camb. 7; 149-158.

42. Prevosti, A. 1972. Chromosomal polymorphism in Drosophila subobscura populations from the Madeira Island; Genet. Iber, 24; 11.

43. Saura, A., S. Lakovaara, J. Lokki and P. Lankinen. 1973. Genetic variation in central and marginal populations of Drosophila subobscura; Hereditas; 33-46.

44. Shaw, Ch. and R. Prasad. 1970. Starch gel electrophoresis of enzymes - a compilation of recipes; Biochem. Genet., 4; 297-320.

45. Shorrocks, B. 1972. INVERTEBRATE TYPES: DROSOPHILA. Ginn and Company limited, London.

46. Sperlich, D. 1959. Experimentelle Beitrage zum Problem des positiven Heterosiseffekts bei der strukturpolymorphen Art Drosophila subobscura; Z. Vererb., 90; 273-287.

47. Sperlich, D. 1961. Untersuchunger über den chromosomalen Polymorphismus einer Population von Drosophila subobscura auf den Liparischen Inseln; Z. Vererbungsl., 92; 74-84.

48. Sperlich, D. and E. Kunze-Muhl. 1963. Der chromosomale Polymorphismus einer Population von Drosophila subobscura auf der Insel Ustica im vergleich mit anderen Insel - und Festlandsstandorten; Z. Vererbungsl., 94; 94-100.

49. Sperlich, D. 1964. Chromosomale Strukturanalyse und Fertilitaespruefung an einer marginal-Population von Drosophila subobscura; Z. indukt. Abstann.-u. Vererblehre, 95; 73-81.

50. Sperlich, D. 1966. Equilibria for inversions induced by X-rays in isogenic strains of Drosophila pseudoobscura; Genetics, 53, No. 5; 835-842.

51. Sperlich, D. and H. Feuerbach-Mravlag. 1974. Epistatic gene interaction, crossing over and linked and unlinked inversions in Drosophila subobscura; Evolution Vol. 28; No. 1.

52. Sperlich, D., H. Feuerbach-Mravlag, P. Lange, A. Michaelidis and A. Pentozos-Daponte. 1977. Genetic load and viability distribution in general and marginal populations of Drosphila subobscura; Gene., 86; 835-848.

53. Stumm-Zollinger, E. 1953. Vergleichende Untersuchungen über die Inversionshaufigkeit bei Drosophila subobscura in Populationen der Schweiz und Südwesteuropas; Z. indukt. Abstamm.-u. Vereb. -Lehre, 85; 382-407.

54. Sturtevant, A.H. 1942. The classification of the genus Drosophila with descriptions of nine new species; Univ. Texas Publ., 4213; 5-51.

55. Watnabe, T., W. Anderson, Th. Dobzhansky and O. Pavlovsky. 1970. Selection in experimental populations of Drosphila pseudoobscura with different initial frequencies of chromosomal variants; Genet. Res., 15; 123-129.

56. Wilson, A.C., V. Sarich and L. Maxson. 1974. The importance of gene arrangement in evolution: Evidence from studies on rates of chromosomal, protein, and anatomical evolution; Proc. Nat. Acad. Sci., 71; 3028-3030.

57. Wilson, A.C. 1975. Social structuring of mammalian populations and rate of chromosomal evolution; Proc. Nat. Acad. Sci., 72; 5061-5062.

58. Zouros, E., C.B. Krimbas, S. Tsakas and M. Loukas. 1974. Genic versus chromosomal variation in natural populations of Drosophila subobscura; Genetics, 78; 1223-1244.

RESUME

Michelle Poulin-Alfeld
351 South 13 East No. 1
Salt Lake City, Utah

On March 10, 1951, I was born in Redwood City, California. As a graduate of Clayton Valley High School, Concord, California in 1969, I entered the University of California, Davis. My Bachelor of Arts Degree in Biology and Zoology was completed at Humboldt State University, California in 1973. I continued there in a Masters of Arts program in Biology under the supervision of Professor T. Kersletter, spending one year at Dundee University, Scotland doing my thesis in Freshwater Ecology (1975).

In the Fall of 1975 I started work under Professor Dr. D. Sperlich at Universität Tuebingen and left in the Fall of 1978 for Salt Lake City, Utah. I am currently teaching at the University of Utah, Department of Continuing Education, and the Museum of Natural History, Salt Lake City, Utah, U.S.A.

Angabe der Lehrer:

Prof.Dr.D.SPERLICH

Prof.Dr.W,SEYFFERT

Prof.Dr.K.WÖHRMANN

Prof.Dr.W.HARDER

Prof.Dr.B.SCHWEMMLE